EXHIBIT 1

From: **Ghassan Saed** gsaed@med.wayne.edu
Subject: Re: SRI abstract
Date: May 10, 2021 at 4:10 PM
To: Robert Morris rmorris@med.wayne.edu
Cc: Ghassan Saed gsaed@med.wayne.edu, Amy Harper aharper4@med.wayne.edu

Thank you!

> On May 9, 2021, at 9:42 PM, Robert Morris <rmorris@med.wayne.edu> wrote:
>
> Nice work Ghassan.
>
> Bob
>
> Sent from my iPhone so excuse the brevity and typos.
>
> > On May 7, 2021, at 4:21 PM, Ghassan Saed <gsaed@med.wayne.edu> wrote:
> >
> > Dear Bob and Amy
> > I have submitted an abstract which is part of our ongoing work with talcum powder and ovarian cancer to SRI. I hope it will get accepted.
> >
> > Please see attached.
> >
> > Dr. Ghassan M. Saed
> > Associate Professor of
> > Gynecologic Oncology
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> > <SRI-21 abstract.pdf>

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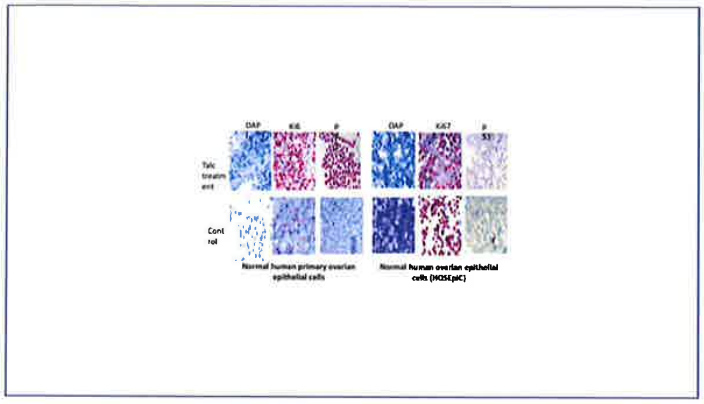
Current Date/Time: 5/7/2021 3:18:02 PM

Talcum powder induces malignant transformation of human primary normal ovarian epithelial cells

Author Block: Ghassan M. Saed*, Amy Harper†, Robert Morris*. *Wayne State University, Detroit, MI, United States.*

Abstract:
Introduction: Molecular and epidemiological studies have demonstrated an association between the genital use of talcum powder and an increased risk of ovarian cancer (OC). Previously we have shown that using an agar transformation assay. The objective of this study is to confirm such an important finding with a different assay.
Methods: Human primary ovarian epithelial cells (HPOE) and ovarian epithelial cells (HOSEpiC) were treated with either 100 ug/ml of talcum powder or titanium dioxide (TiO2) as a particulate control for 72 hours before assessment of p53 and Ki67 expression with immunohistochemistry (IHC).
Results: Focal p53 nuclear staining indicating wild type p53 expression was observed in both cell lines before treatment. After treatment of cells with talcum powder 100 ug/ml for 72 hours, diffused "in-block" nuclear staining was observed indicating p53 mutated form. Additionally, talcum powder treatment increased the proliferation index (PI) in both cell lines. The baseline PI for HPOE and HOSEpic cells was 50 and 70% respectively. The PI was significantly increased to 90% in both cell lines (Figure 1).
Conclusion: Exposure to talcum powder induces malignant transformation in ovarian epithelial cells. These findings represent a direct effect of talcum powder exposure and further supports previous studies demonstrating a link between the genital use of talcum powder and increased risk of OC.



Category (Complete): 06.1-Cancer Biology
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My submitted abstract(s) contains original data, written in standard scientific form, complete with numeric values and statistical analyses when appropriate.: Yes
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I will comply with the SRI Withdrawal Policy. : True
Translational Value: Yes
Please describe the translational relevance below : the fact that genital talcum powder use is linked to ovarian cancer risk

Keyword (Complete): ovarian cancer ; Talcum powder ; immunohistochemistry
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ovarian epithelial cells

Ghassan M. Saed, Amy K. Harper, Robert Morris

Department of Obstetrics and Gynecology, Wayne State University, and Karmanos cancer Institute, Detroit, MI, United States.

Background

Molecular and epidemiological studies have demonstrated an association between the genital use of talcum powder and an increased risk of ovarian cancer (1-4). Several *in vitro* studies have demonstrated a biologic effect when cells in culture are exposed to talcum powder (1-4). In support of these findings, we have recently delineated the molecular basis of the association of talcum powder use with increased risk of ovarian cancer (5). More importantly, we have recently shown that exposure of normal ovarian epithelial cells to talcum powder induced transformation of these cells, using an agar transformation assay.

Objective

We sought to confirm that exposure of normal ovarian epithelial cells to talcum powder induces transformation of these cells with a different assay.

Methods

Human primary ovarian epithelial cells (HPOE) and ovarian epithelial cells (HOSEpiC) were treated with either 100 ug/ml of talcum powder or titanium dioxide (TiO2) as a particulate control for 72 hours before assessment of p53 and Ki67 expression with immunohistochemistry (IHC).

Results

Focal p53 nuclear staining indicating wild type p53 expression was observed in both cell lines before treatment. After treatment of cells with talcum powder 100 ug/ml for 72 hours, diffused "in-block" nuclear staining was observed indicating p53 mutated form. Additionally, talcum powder treatment increased the proliferation index (PI) in both cell lines. The baseline PI for HPOE and HOSEpic cells was 50 and 70% respectively. The PI was significantly increased to 90% in both cell lines (Figure 1).

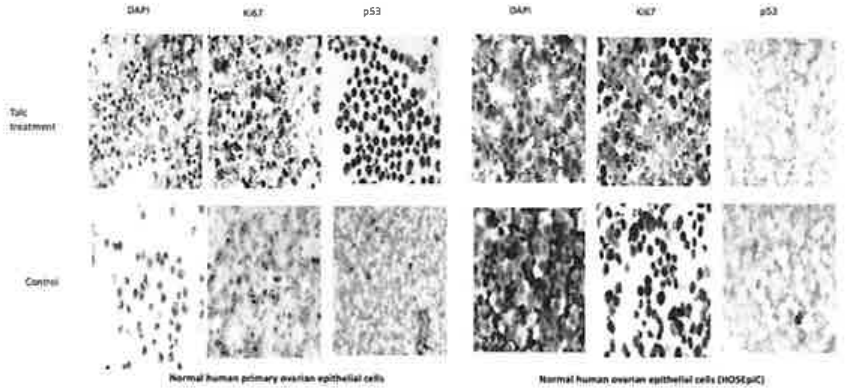

Figure 1: Immunohistochemistry staining for p53 and Ki67 in two normal human ovarian epithelial cells with and without Talcum powder (100 ug/ml) treatment for 72 hours. Slides were reviewed by two pathologists.

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Dr. Saed has s
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From: **International Journal of Gynecological Cancer** onbehalfof@manuscriptcentral.com
Subject: IJGC Decision
Date: March 2, 2021 at 9:47 AM
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[EXTERNAL]

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Manuscript Number: ijgc-2021-002562
Title: Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells

Dear Dr. Saed,

Thank you for submitting your manuscript to International Journal of Gynecological Cancer. The Editorial Team has reviewed your recent submission and, unfortunately, your submission is not considered acceptable for publication.

The journal receives many submissions and due to space limitations are only able to accept a small portion of them. The specific comments of the Editorial Team are included below. Please note that all decisions are final and that the journal does not accept rebuttals.

On initial evaluation of the manuscript, the Editorial team considers that the manuscript addresses a topic viewed outside the scope of the journal and thus, unfortunately, the manuscript will not be considered for further review. Although the manuscript may be considered of related interest, we have collectively determined that the subject addressed in such manuscript is not appropriate for the readership of our journal.

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With Kind Regards,
Dr. Pedro T. Ramirez
Editor-in-Chief
International Journal of Gynecological Cancer

Dear sir/madam,

Please consider our manuscript "Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells" for publication in your journal. We have previously published that Talcum powder induces an inflammatory/oxidative stress profile in normal epithelial ovarian cells similar to that seen in ovarian cancer cells. Here we are excited to demonstrate that exposure to talcum powder induces malignant transformation in normal ovarian epithelial cells but not in normal peritoneal fibroblasts. This finding is intriguing, and work is currently ongoing in our laboratory to understand the mechanism.

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Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells

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Abstract

Background: Several studies have linked perineal use of talcum powder to increased risk of ovarian cancer (OC). Here, we determined that exposure to talcum powder induces malignant transformation in human normal ovarian cells.

Methods: Human primary ovarian epithelial cells (HPOE), ovarian epithelial cells (HOSEpiC), and primary fibroblasts (NF) were treated with either 100 or 500 ug/ml of talcum powder or titanium dioxide (TiO_2) as a particulate control for 72 hours before assessment with a cell transformation assay and p53 and Ki67 immunohistochemistry.

Results: Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in a dose dependent manner in ovarian cell lines. No colonies formed in the untreated ovarian cells or control ovarian cells (TiO_2 treated) at either dose. There were no colonies formed in talc treated NF cells. Transformed ovarian cells were increased by 11% and 20% in HPOE and 24% and 40% in HOSEpic cells for talcum powder 100 and 500 ug/ml doses, respectively ($p<0.05$). There were no detectible transformed cells when cells were treated with TiO_2. Importantly, p53 mutant type as well as increased expression of Ki67 were detected in HPOE and HOSEpic cells when exposed to talcum powder.

Conclusion: Exposure to talcum powder induces malignant transformation in ovarian epithelial cells but not in NF cells. These findings represent a direct effect of talcum powder exposure that is specific to normal ovarian cells and further supports previous studies demonstrating an association between the genital use of talcum powder and an increased risk of OC.

Introduction

Ovarian cancer is a gynecologic malignancy that ranks fifth in cancer deaths among women in United States [1]. Epithelial ovarian cancer (EOC) presents with various histopathology, molecular biology, and clinical outcome and is therefore considered a heterogeneous disease [2]. The pathogenesis of EOC is strongly associated with oxidative stress and inflammation [3-5].

Epithelial ovarian cancer cells manifest a persistent pro-oxidant state that has been demonstrated in vitro and is also enhanced in chemoresistant EOC cells [3, 4]. Attenuation of the pro-oxidant state with antioxidants/scavengers has been shown in vitro to selectively induce apoptosis in EOC cells indicating a potential therapeutic value [6, 7]. Talcum Powder has been shown to induce oxidative stress and cell proliferation and to decrease apoptosis in normal ovarian cells and thus may play an important role in the pathogenesis of EOC [8].

The association between genital use of talcum powder and risk of ovarian cancer have been described in numerous studies [8-11]. Several meta-analyses have demonstrated a statistically significant increased risk of ovarian cancer with the genital use of talcum powder [11-13]. In addition, several animal studies have reported that talcum powder causes inflammation and oxidative stress [14-16]. Several *in vitro* studies have demonstrated a biologic effect when cells in culture are exposed to talcum powder [17-21].

In support of these previous findings, we have recently delineated the molecular basis of the association of talcum powder use with increased risk of ovarian cancer [8]. The specific mechanism by which talcum powder exposure causes ovarian cancer has not

been definitively established. Here we clearly demonstrate that exposure to talcum powder induces malignant transformation in human primary normal ovarian epithelial cells and thus, providing a mechanism for the increased risk of ovarian cancer with the genital use of talcum powder.

Methods

Normal human primary ovarian epithelial cells (HPOE): Cells were received at passage 3 (Cell Biologics, Chicago, IL) cryo-preserved in vials containing at least $0.5x10^6$ cells per ml. Cells were grown in gelatin pre-coated T25 flasks for 2 min and incubated in *Cell Biologics'* Culture Complete Growth Medium. Cells were expanded for 2-4 passages at a split ratio of 1:2 under the cell culture conditions as specified by *Cell Biologics*. Human Epithelial Cell Medium is a complete medium designed for the culture of human epithelial cells. It was tested and optimized with epithelial cell growth and proliferation in vitro. Cells were incubated at 37 °C with 5% CO_2 and 95% air.

Normal human ovarian epithelial cells (HOSEpiC): Cells were purchased from ScienCell Research Laboratories, Inc, Carlsbad, California. HOSEpiC cells were isolated from human ovary. HOSEpiC cells were received cryopreserved at passage one in frozen vials, each vial contains 5×10^5 cells in 1 ml volume. Cells were further expanded for 2-3 passages in Ovarian Epithelial Cell Medium (OEpiCM, Cat. #7311). Cells were incubated at 37°C with 5% CO_2 and 95% air.

Human normal primary peritoneal fibroblasts: This fibroblast cell line has been extensively characterized in previous studies [22]. Cells were grown in Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, CA), with 10 % fetal bovine serum (FBS,

Innovative Research, Novi, MI) and penicillin/streptomycin (Fisher Scientific, Waltham, MA) as we have previously described [22]. Cells were incubated at 37 °C with 5% CO_2 and 95% air.

Talcum powder treatment: Talcum baby powder (Johnson & Johnson, New Brunswick, NJ, #30027477, Lot#13717RA) or control particles, Titanium dioxide (TiO_2, Spectrum Chemical Corp, Lot No. 2EB0148) were used to treat cells. Talcum powder or TiO_2 were suspended in PBS (Stock solution of 50 mg/ml) and sonicated 3 times for 1 minute each with Sonic Dismembrator (Fischer Scientific, Model 100). Stock solutions were filtered through 30 µm nylon mesh filters. No visible loss of material has observed. Cells were seeded in 100 mm Petri cell culture dishes (1×10^6) and were treated 24 hours later in duplicate in a fresh media with 100 or 500 µg/ml of talc or titanium dioxide (TiO_2) for 72 hours. Control: cells (30K) with media only and Negative control: cells (30K) with media and PBS. No cell death was observed after 72 hours in culture in control or treated cells. Titanium dioxide, a naturally occurring particle, has been classified in humans and animals as biologically inert [19, 23]. Titanium dioxide particles are produced and used as fine (~ 0.1-2.5 µm) and nanosize (<0.1 µm) particles [23]. In this study, we used TiO_2 as a particulate control to exclude the effect of material size. Culture plates were washed several times to remove residual particles and collected by trypsin in fresh media. Cells were counted and their concentration was adjusted with fresh media to 1.5×10^6 cells/ml.

Cells were now ready to be assessed with cell transformation assay (colorimetric), according to the manufacturer protocol (Abcam-235698, Cambridge, MA). The 100 and 500 ug/ml doses were chosen based on our previous studies which showed talcum

116 powder to induce changes in redox balance of cells at the molecular level [8]. The
117 experiments were repeated 3 times with a fresh solution of talcum powder and TiO_2. This
118 assay is more stable, faster and more sensitive than the traditional Soft-Agar Assay.
119 Traditional assays require 3-4 weeks of incubation and inconsistent due to independent
120 counting. An additional advantage of this assay is it's linear range from 10,000-400,000
121 cells.
122
123 A Cell-dose curve was established as described in the manufacturer's protocol. Briefly,
124 we used cells (5.34 x 10^5 cells/ml) were suspended in 1X DMEM/10% FBS medium. Cells
125 were diluted into seven serial dilutions in a 1.5 mL centrifuge tubes. Serial dilution was
126 performed using an 8 channel multi pipette by adding 150 µl of media to each well of a
127 96 well microplate. A 150 µl aliquot of the 5.34 x10^5 cells/ml (80 x10^3 cells) was added to
128 the wells of the first duplicate row. A 150 µl aliquot from the first duplicate row was
129 removed and added to the next well and mixed. The process was repeated until the seven
130 serial dilutions were obtained. The final well was blank with media only and no cells. A 35
131 µl aliquot of 1X DMEM/10% FBS and 15 µl of WST working solution were then added into
132 each well and incubate at 37° C for 4 hours. The absorbance was measured by a
133 microtiter plate reader at 450 nm (Figure 1).
134
135 Agarose and WST working solutions were prepared as described in the kit information
136 sheet (Abcam-235698, Cambridge, MA). The base agarose mix was added into the
137 required wells in a 96 well plate and kept for 15 minutes at 4°C to solidify the agarose. A
138 top agarose layer stock solution was prepared by using talcum powder or TiO_2 treated

stock cell solution of 1.5 X 10^6 cells/ml (30,000 cells per well, which is within the recommended range of the assay) in 1X DMEM/10% FBS medium. The agarose-cell mix was added into every well of a 96 well plate previously holding the solidified base agarose layer and placed at 4°C for 10 minutes to solidify the layer. After placing the plate for 10 min at 37°C, 1X DMEM/10% FBS medium was added to all the wells and incubated at 37°C for 6-8 days. On the last day the upper medium on the top agarose layer was cautiously removed by pipetting. A 1X DMEM/10% FBS and WST working solution was added into each well, incubated for 4 hours at 37°C. The absorbance was measured by a microtiter plate reader at 450 nm. Colonies of transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase Contrast Microscope with an Axio camera.

Immunohistochemistry (IHC) staining and scoring: The IHC panel consisted of antibodies against p53 and Ki-67. The primary antibodies, suppliers, and staining conditions are listed below.

Antibody	Clone	Source	Detection System	Dilution
P53	DO-7	Ventana	Ventana ultraView DAB	1:500
Ki-67	Mib1	Ventana	Ventana ultraView DAB	1:2000

Cytospin slides were prepared from cells and stained using immunoperoxidase labeling performed with the automated XT iVIEW DAB V.1 procedure on the Ultra BenchMark XT IHC/ISH Staining Module, Ventana with anti-p53 (clone DO-7 prediluted, Ventana). Antigen retrieval was carried out with CC1, pH 8.0 (Ventana). Sections were incubated with primary antibodies for 36 min at 37°C. All slides were reviewed by two pathologists (Drs Ali and Alrajjal). Cases with discordant Ki-67 estimated results underwent a consensus review at a double-headed microscope. Diffuse "in-block" nuclear staining or

complete negative staining with p53 was considered a positive reaction indicating mutated p53 status. Focal nuclear staining is consistent with "wild type" p53 and considered negative. The Proliferation Index (PI) was assessed qualitatively using Ki-67-stained slides and classified as high PI (>50% positive cells) or low PI (<50% positive cell).

Statistical Analysis: We performed ANOVAs with Tukey post hoc tests to evaluate the difference between the three groups (no treatment control, talcum powder treatment and TiO2 treatment). The values were expressed as mean ± standard deviation. We used SPSS v24 for Windows (SPSS, Chicago, Illinois); a $p<0.05$ defined significance.

Results

Treatment with talcum powder significantly increased the number of transformed normal epithelial ovarian cells by 11% and 20% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, $p<0.05$). Likewise, but to a greater extent, treatment with talcum powder significantly increased the number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, $p<0.05$). Talcum powder had no detectable transformation effect on normal peritoneal fibroblasts at either dose (Figure 2). There was no significant difference between the no treatment control and the two doses of TiO2 treatment control group (Figure 2, $p>0.05$).

It is known that cancer cells are able to grow in culture without the need for matrix attachment. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in normal epithelial ovarian cell lines in a dose dependent

manner (Figure 3). There were no colonies formed in talcum powder treated normal fibroblasts (Figure 3). There were no colonies formed in either untreated ovarian cells or control ovarian cells at either dose. There were no detectible transformed cells when cells were treated with the particulate control, TiO_2.

To confirm malignant cell transformation observed with the cell transformation assay used in this study we performed IHC on the normal human primary ovarian epithelial (HPOE) and normal human ovarian epithelial cells (HOSEpiC) cells staining for p53 and Ki67. Focal p53 nuclear staining indicating wild type p53 expression was observed in cells before treatment. After treatment of cells with talcum powder 100 ug/ml for 72 hours, diffused "in-block" nuclear staining was observed indicating p53 mutated form (Figure 4). Additionally, talcum powder treatment increased the proliferation index (PI) in both cell lines. The baseline PI for HPOE and HOSEpic cells was 50 and 70% respectively. The PI was significantly increased to 90% in both cell lines (Figure 4).

Discussion

This is the first study to directly show that exposure to talcum powder induces malignant transformation in ovarian epithelial cells. The ability of talcum powder exposure to induce transformation appears to be specific to ovarian cells as it did not induce transformation in peritoneal fibroblasts. (Figure 3).

The link between talcum powder exposure and ovarian cancer have been supported by the harmful biological effects reported in various cell culture studies [8, 14, 17-21, 24, 25].

Macrophage activation and inflammatory response to talcum powder were suggested as a link to increased risk of ovarian cancer [14, 18]. Macrophages exposed to nano-talc manifested increased levels in inflammatory markers, TNF-alpha, IL-1beta and IL-6 as well as constituent phosphorylation of both p38 and ERK1/2 pathways [18]. p38 MAPK signaling pathway are known to be associated with cisplatin-resistant ovarian cancer [26]. Exposure of macrophages to talc and estradiol has led to increased production of reactive oxygen species and changes in expression of macrophage genes that play a role in cancer development and immunosurveillance [24]. These studies have also shown that ovarian cancer cells were present in larger numbers after co-culture with macrophages exposed to talc powder when in the presence of estradiol [24].

Oxidative stress have been implicated in the pathogenesis of ovarian cancer, specifically, by increased expression of several key pro-oxidant enzymes in EOC tissues and cells as compared to normal cells [27]. Talcum powder exposure was shown to induce molecular changes in redox enzymes in normal ovarian cells similar to those known for ovarian cancer [3, 8]. In all talc-treated cells, there was a significant dose-dependent increase in key prooxidants with a concomitant decrease in key antioxidants enzymes. Remarkably, talcum powder exposure induced specific point mutations that are known to alter the activity in some of these key enzymes. The mechanism by which talcum powder alters the cellular redox and inflammatory balance involves the induction of specific mutations in key oxidant and antioxidant enzymes that correlate with alterations in their activities. [8].

Ovarian cancer cells was shown to manifest increased cell proliferation and decreased apoptosis, a hallmark of malignant cells, as compared to normal ovarian cells [27]. Indeed, talcum powder further enhanced cell proliferation and inhibited apoptosis in EOC cells, but more importantly in normal ovarian cells, suggesting talc is a stimulus to the development of the oncogenic phenotype [8]. Furthermore, CA-125, a membrane-bound and secreted protein, has been established as a biomarker for disease progression and response to ovarian cancer treatment [28]. CA-125 was significantly increased to values approaching clinical significance (35 U/ml in postmenopausal women) in talc treated normal ovarian cells [8, 28]. Thus, these findings confirmed the inflammatory/redox stress effects of talcum powder exposure to normal ovarian cells and indicated that this stress is a key mechanism in the malignant transformation of these cells.

The dose and time of talcum powder exposure in cell culture experiments used in this study was based on previous studies [8]. These doses are not intended to represent a typical dose when applied to the genital area in women over time. Despite this limitation, the development and use of in vitro models has been valuable in the advancement of research and knowledge on cancer pathogenesis [29]. The cellular transformation demonstrated in this study was significant and informative.

Anchorage-independent growth is one of the hallmarks of cell transformation and is accepted to be the most accurate and stringent in vitro assay for detecting malignant transformation of cells [30, 31]. The soft agar colony formation assay used in this study is widely accepted and used to evaluate cellular transformation *[30, 31]*. The Cell

Transformation Assay Kit is faster, stable, more sensitive, and has a wide linear range (10,000-400,000) cells than the traditional Soft-Agar Assay. Therefore, in this study we used 30,000 of talcum powder and TiO_2 treated cells as well as control cells to stay within the recommended number of cells. The assay utilizes the conversion of tetrazolium salt to formazan by mitochondrial dehydrogenases which is directly proportional to the number of living transformed cells (Figure 2).

Tumor suppressor p53 gene mutations are frequently seen in ovarian cancers and can be used as a biomarker to differentiate low from high grade serous ovarian carcinomas. The methods used for the assessment of p53 (mutant vs. wild type) and Ki67 expression in this study is identical to the methods used in clinical pathology laboratories for the diagnosis of the different subtypes of ovarian cancer. The slides were scored and interpreted independently by two pathologists. Mutant p53 along with increased Ki67 expression were detected in both HPOE and (HOSEpiC) ovarian cells treated with 100 ug/ml talcum powder for 72 hours (Figure 4). These findings supported the malignant transformation of normal ovarian cells seen in the agar transformation assay (Figure 3).

This study clearly demonstrate that talcum powder exposure induced malignant transformation of normal ovarian cells in culture which adds to the strong evidence of a causal relationship between the genital use of talcum powder and ovarian cancer. Therefore, we consider that future studies should aim to evaluate this finding utilizing animal models.

In conclusion, the ability of talcum powder exposure to induce malignant transformation appears to be specific to ovarian cells as it did not induce transformation in normal peritoneal fibroblasts. Further investigation to understand this specific effect of talcum powder on the ovaries is needed.

Acknowledgment: We acknowledge Dr. Ruba Ali-Fahmi and Dr. Ahmad Alrajjal from the department of pathology who helped with immunohistochemistry of p53 and Ki67.

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Figure Legends

Figure 1: Human primary normal ovarian epithelial cell-dose curve. The cell dose curve was established as described in methods using a serial dilution of cells.

Figure 2: Equal numbers (30K) of human primary normal ovarian epithelial cells (HPOE), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) were seeded for the cell transformation assay as described in methods. After 6 days, the cell number were measured. Standard and samples readings were taken 4 hours after adding WST working solution. Control: cells (30K) with media only.

Figure 3: Images of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) treated with 100 and 500 ug/ml of talcum powder, after 6 days of culture. Colonies of transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase Contrast Microscope with an Axio camera.

Figure 4: Immunohistochemistry staining for p53 and Ki67 in two normal human ovarian epithelial cells with and without Talcum powder (100 ug/ml) treatment for 72 hours. Slides were reviewed by two pathologists. Diffuse nuclear staining or complete negative staining with p53 is considered a positive reaction indicating mutated p53 status were observed in cells treated with talcum powder. Focal nuclear staining is consistent with wild type p53

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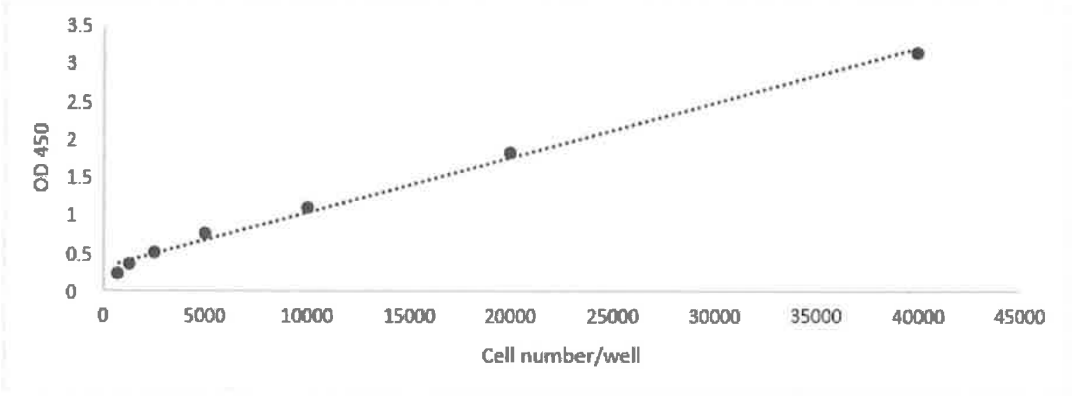

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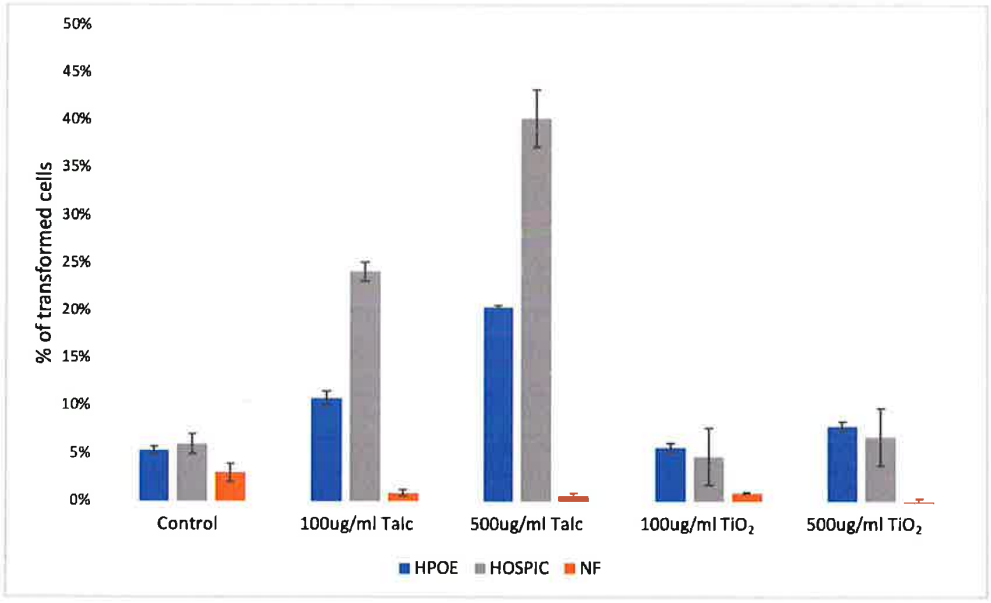

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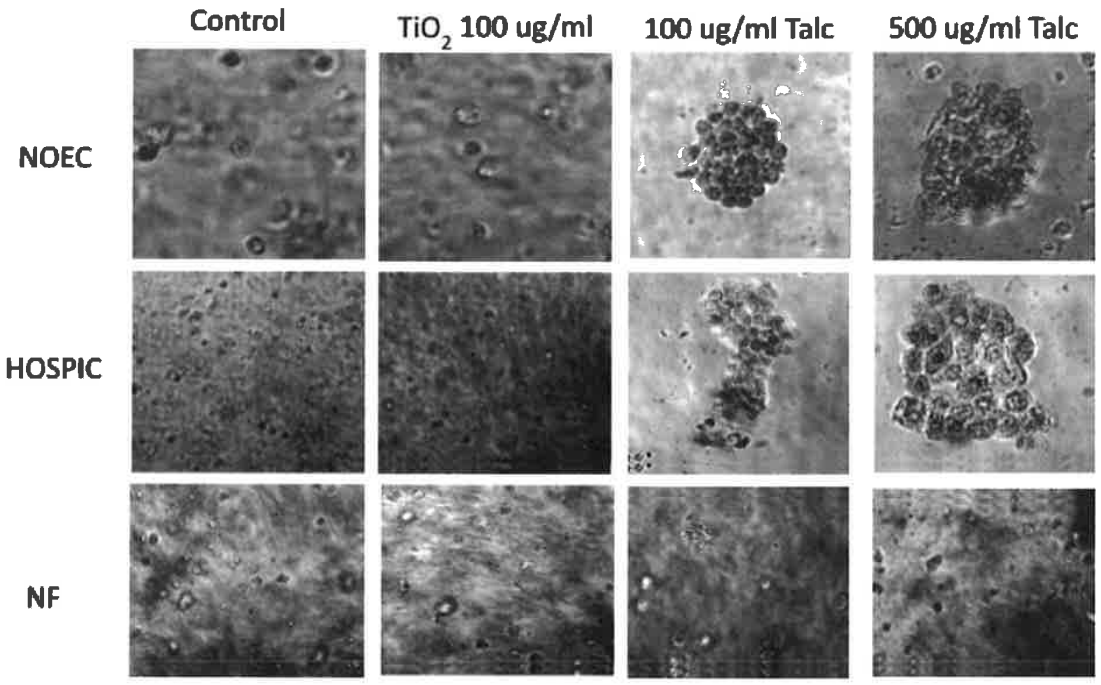

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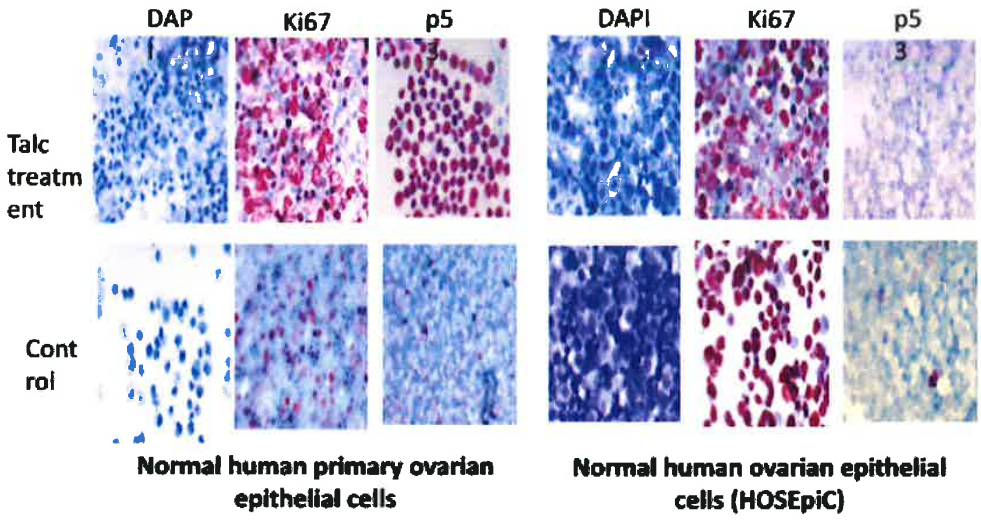

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HIGHLIGHTS

- Several studies have linked perineal use of talcum powder to increased risk of ovarian cancer.
- Several in vitro studies have demonstrated a biologic effect when cells in culture are exposed to talcum powder.
- Exposure to talcum powder induces malignant transformation in human ovarian epithelial cells but not in normal peritoneal fibroblasts.

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Running title: Talcum powder use and risk of ovarian cancer

Title: Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells

Amy K. Harper, M.D.[1,2], Xin Wang, M.S. [1], Rong Fan, M.S.[1], Nicole M. Fletcher, Ph.D.[1], Robert T. Morris, M.D.[2], *Ghassan M. Saed, Ph.D.[1,2]

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Capsule: Talcum powder induces malignant transformation in ovarian epithelial cells.

Abstract

Purpose: To study whether exposure to talcum powder induces malignant transformation in human normal ovarian cells.

Design: Prospective experimental study.

Setting: University medical center

Patient(s): Cell lines from normal peritoneum and ovaries

Intervention(s): Human primary ovarian epithelial cells (HPOE), ovarian epithelial cells (HOSEpiC), and primary fibroblasts (NF) were treated with either 100 or 500 ug/ml of talcum powder or titanium dioxide (TiO_2) as a particulate control for 72 hours.

Main outcome measure(s): Transformation of cells was assessed with cell transformation assay and immunohistochemistry of p53 and Ki67.

Result(s): Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in a dose dependent manner in ovarian cell lines. No colonies formed in the untreated ovarian cells or control ovarian cells (TiO_2 treated) at either dose. There were no colonies formed in talc treated NF cells. Transformed ovarian cells were increased by 11% and 20% in HPOE and 24% and 40% in HOSEpic cells for talcum powder 100 and 500 ug/ml doses, respectively ($p<0.05$). There were no detectible transformed cells when cells were treated with TiO_2. Importantly, p53 mutant type as well as increased expression of Ki67 were detected in HPOE and HOSEpic cells when exposed to talcum powder.

Conclusion(s): Exposure to talcum powder induces malignant transformation in ovarian epithelial cells but not in NF cells. These findings represent a direct effect of talcum powder exposure that is specific to normal ovarian cells and further supports previous studies demonstrating an association between the genital use of talcum powder and an increased risk of OC.

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Introduction

Ovarian cancer is a gynecologic malignancy that ranks fifth in cancer deaths among women in United States [1]. Epithelial ovarian cancer (EOC) presents with various histopathology, molecular biology, and clinical outcome and is therefore considered a heterogeneous disease [2]. The prognosis of EOC remains poor, with a 5-year survival rate of 50% in advanced stage [3]. This is largely due to the lack of early warning symptoms, screening methods, and the eventual development of chemoresistance [3].

The pathogenesis of EOC is strongly associated with oxidative stress and inflammation [4-8]. Epithelial ovarian cancer cells manifest a persistent pro-oxidant state that has been demonstrated in vitro and is also enhanced in chemoresistant EOC cells [4, 6]. Attenuation of the pro-oxidant state with antioxidants/scavengers has been shown in vitro to selectively induce apoptosis in EOC cells indicating a potential therapeutic value [5, 9, 10]. Talcum Powder has also been shown to induce oxidative stress and cell proliferation and to decrease apoptosis in ovarian cancer cells and in normal ovarian cells [11].

The association between genital use of talcum powder and risk of ovarian cancer have been described in numerous studies [11-18]. Several meta-analyses have demonstrated a statistically significant increased risk of ovarian cancer with the genital use of talcum powder [16, 19, 20]. In addition, several animal studies have reported that talcum powder causes inflammation and oxidative stress [21-25]. Several *in vitro* studies have demonstrated a biologic effect when cells in culture are exposed to talcum powder [26-31]. In support of these previous findings, we have recently delineated the molecular basis of the association of talcum powder use with increased risk of ovarian cancer [11]. Despite these concerns, the specific mechanism by which talcum powder exposure causes ovarian cancer has not been definitively established.

Here we clearly demonstrate that exposure to talcum powder induces malignant transformation in human primary normal ovarian epithelial cells and thus, providing a mechanism for the increased risk of ovarian cancer with the genital use of talcum powder.

Material and Methods

Cell lines:

Normal human primary ovarian epithelial cells (HPOE): Cells were purchased from Cell Biologics, Chicago, IL. Cells were received at passage 3 cryo-preserved in vials containing at least $0.5x10^6$ cells per ml. Cells were grown in gelatin pre-coated T25 flasks for 2 min and incubated in *Cell Biologics'* Culture Complete Growth Medium.. Cells were expanded for 2-4 passages at a split ratio of 1:2 under the cell culture conditions as specified by *Cell Biologics.* Human Epithelial Cell Medium is a complete medium designed for the culture of human epithelial cells. It was tested and optimized with epithelial cell growth and proliferation in vitro.

Cells were incubated at 37 °C with 5% CO_2 and 95% air in a humidified incubator. The medium consists of 500 ml of basal medium (containing essential and non-essential amino acids, vitamins, organic and inorganic compounds, hormones, growth factors, trace minerals), supplemented with epithelial cell growth supplement, antibiotics, and fetal bovine serum.

Normal human ovarian epithelial cells (HOSEpiC): Cells were purchased from ScienCell Research Laboratories, Inc, Carlsbad, California. HOSEpiC cells were isolated from human ovary. HOSEpiC cells were received cryopreserved at passage one in frozen vials, each vial contains >5 x 10^5 cells in 1 ml volume. HOSEpiC were further expanded for 2-3 passages under the conditions provided by ScienCell Research Laboratories. Cells were grown in Ovarian Epithelial Cell Medium (OEpiCM, Cat. #7311). Cells were incubated at 37°C with 5% CO_2 and 95% air in a humidified incubator.

Human normal primary peritoneal fibroblasts: Cells were isolated and cultured as we have previously described [32, 33]. The fibroblast cell line has been extensively characterized in previous studies and has been shown to be pure and solely fibroblast cells [32, 33]. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, CA), supplemented with 10 % fetal bovine serum (FBS, Innovative Research, Novi, MI) and penicillin/streptomycin (Fisher Scientific, Waltham, MA) as we have previously described [32, 33]. Cells were incubated at 37 °C with 5% CO_2 and 95% air in a humidified incubator.

Talcum powder treatment: Talcum baby powder (Johnson & Johnson, New Brunswick, NJ, #30027477, Lot#13717RA) or control particles, Titanium dioxide (TiO_2, Spectrum Chemical Corp, Lot No. 2EB0148) were used to treat cells. Talcum powder or TiO_2 were suspended in PBS (Stock solution of 50 mg/ml) and sonicated 3 times for 1 minute each with Sonic Dismembrator

(Fischer Scientific, Model 100). Stock solutions were filtered through 30 µm nylon mesh filters. No visible loss of material has observed. Cells were seeded in 100 mm Petri cell culture dishes (1 x 10^6) and were treated 24 hours later in duplicate in a fresh media with 100 or 500 µg/ml of talc or titanium dioxide (TiO_2) for 72 hours. Control: cells (30K) with media only and Negative control: cells (30K) with media and PBS. No cell death was observed after 72 hours in culture in control or treated cells. Titanium dioxide, a naturally occurring particle, has been classified in humans and animals as biologically inert [28, 34]. Titanium dioxide particles are produced and used as fine (~ 0.1-2.5 µm) and nanosize (<0.1 µm) particles [34]. In this study, we used TiO_2 as a particulate control to exclude the effect of material size. Culture plates were washed several times to remove residual particles and collected by trypsin in fresh media. Cells were counted and their concentration was adjusted with fresh media to 1.5 X 10^6 cells/ml.

Cells were now ready to be assessed with cell transformation assay (colorimetric), according to the manufacturer protocol (Abcam-235698, Cambridge, MA). The 100 and 500 ug/ml doses were chosen based on our previous studies which showed talcum powder to induce changes in redox balance of cells at the molecular level [11]. The experiments were repeated 3 times with a fresh solution of talcum powder and TiO_2. This assay is more stable, faster and more sensitive than the traditional Soft-Agar Assay. Traditional assays require 3-4 weeks of incubation and inconsistent due to independent counting. An additional advantage of this assay is it's linear range from 10,000-400,000 cells.

A Cell-dose curve was established as described in the manufacturer's protocol. Briefly, we used cells (5.34 x 10^5 cells/ml) were suspended in 1X DMEM/10% FBS medium. Cells were diluted into seven serial dilutions in a 1.5 mL centrifuge tubes. Serial dilution was performed

using an 8 channel multi pipette by adding 150 µl of media to each well of a 96 well microplate. A 150 µl aliquot of the 5.34 $x10^5$ cells/ml (80 $x10^3$ cells) was added to the wells of the first duplicate row. A 150 µl aliquot from the first duplicate row was removed and added to the next well and mixed. The process was repeated until the seven serial dilutions were obtained. The final well was blank with media only and no cells. A 35 µl aliquot of 1X DMEM/10% FBS and 15 µl of WST working solution were then added into each well and incubate at 37° C for 4 hours. The absorbance was measured by a microtiter plate reader at 450 nm (Figure 1).

Agarose and WST working solutions were prepared as described in the kit information sheet (Abcam-235698, Cambridge, MA). The base agarose mix was added into the required wells in a 96 well plate and kept for 15 minutes at 4°C to solidify the agarose. A top agarose layer stock solution was prepared by using talcum powder or TiO_2 treated stock cell solution of 1.5 X 10^6 cells/ml (30,000 cells per well, which is within the recommended range of the assay) in 1X DMEM/10% FBS medium. The agarose-cell mix was added into every well of a 96 well plate previously holding the solidified base agarose layer and placed at 4°C for 10 minutes to solidify the layer. After placing the plate for 10 min at 37°C, 1X DMEM/10% FBS medium was added to all the wells and incubated at 37°C for 6-8 days. On the last day the upper medium on the top agarose layer was cautiously removed by pipetting. A 1X DMEM/10% FBS and WST working solution was added into each well, incubated for 4 hours at 37°C. The absorbance was measured by a microtiter plate reader at 450 nm. Colonies of transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase Contrast Microscope with an Axio camera.

Immunohistochemistry (IHC) staining and scoring: The IHC panel consisted of antibodies against p53 and Ki-67. The primary antibodies, suppliers, and staining conditions are listed below.

Antibody	Clone	Source	Detection System	Dilution
P53	DO-7	Ventana	Ventana ultraView DAB	1:500
Ki-67	Mib1	Ventana	Ventana ultraView DAB	1:2000

Cytospin slides were prepared from cells and stained using immunoperoxidase labeling performed with the automated XT iVIEW DAB V.1 procedure on the Ultra BenchMark XT IHC/ISH Staining Module, Ventana with anti-p53 (clone DO-7 prediluted, Ventana). Antigen retrieval was carried out with CC1, pH 8.0 (Ventana). Sections were incubated with primary antibodies for 36 min at 37°C. All slides were reviewed by two pathologists (Drs Ali and Alrajjal). Cases with discordant Ki-67 estimated results underwent a consensus review at a double-headed microscope. Diffuse "in-block" nuclear staining or complete negative staining with p53 was considered a positive reaction indicating mutated p53 status. Focal nuclear staining is consistent with "wild type" p53 and considered negative. The Proliferation Index (PI) was assessed qualitatively using Ki-67-stained slides and classified as high PI (>50% positive cells) or low PI (<50% positive cell).

Statistical Analysis: We performed ANOVAs with Tukey post hoc tests to evaluate the difference between the three groups (no treatment control, talcum powder treatment and TiO2 treatment). The values were expressed as mean ± standard deviation. We used SPSS v24 for Windows (SPSS, Chicago, Illinois); a $p<0.05$ defined significance.

Results

Treatment with talcum powder significantly increased the number of transformed normal epithelial ovarian cells by 11% and 20% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, $p<0.05$). Likewise, but to a greater extent, treatment with talcum powder significantly increased the number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, $p<0.05$). Talcum powder had no detectable transformation effect on normal peritoneal fibroblasts at either dose (Figure 2). There was no significant difference between the no treatment control and the two doses of TiO2 treatment control group (Figure 2, $p>0.05$).

It is known that cancer cells are able to grow in culture without the need for matrix attachment. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in normal epithelial ovarian cell lines in a dose dependent manner (Figure 3). There were no colonies formed in talcum powder treated normal fibroblasts (Figure 3). There were no colonies formed in either untreated ovarian cells or control ovarian cells at either dose. There were no detectible transformed cells when cells were treated with the particulate control, TiO_2.

To confirm malignant cell transformation observed with the cell transformation assay used in this study we performed IHC on the normal human primary ovarian epithelial (HPOE) and normal human ovarian epithelial cells (HOSEpiC) cells staining for p53 and Ki67. Focal p53 nuclear staining indicating wild type p53 expression was observed in cells before treatment. After treatment of cells with talcum powder 100 ug/ml for 72 hours, diffused "in-block" nuclear

staining was observed indicating p53 mutated form (Figure 4). Additionally, talcum powder treatment increased the proliferation index (PI) in both cell lines. The baseline PI for HPOE and HOSEpic cells was 50 and 70% respectively. The PI was significantly increased to 90% in both cell lines (Figure 4).

Discussion

This is the first study to directly show that exposure to talcum powder induces transformation in normal human ovarian epithelial cells. The ability of talcum powder exposure to induce transformation appears to be specific to ovarian cells as it did not induce transformation in human normal peritoneal fibroblasts. (Figure 3). The reason for this specific effect of talcum powder on the ovaries in still under investigation.

The dose and time of talcum powder exposure in cell culture experiments used in this study was based on previous studies [11]. These doses are not intended to represent a typical dose when applied to the genital area in women over time. Despite this limitation, the development and use of in vitro models has been valuable in the advancement of research and knowledge on cancer pathogenesis [35]. The cellular transformation demonstrated in this study was significant and informative.

The soft agar in vitro colony formation assay is widely accepted and used to evaluate cellular transformation *[36, 37]* . Anchorage-independent growth is one of the hallmarks of cell transformation and is accepted to be the most accurate and stringent in vitro assay for detecting malignant transformation of cells [36, 37]. In this study we used Cell Transformation Assay Kit,

which is faster, stable, and more sensitive than the traditional Soft-Agar Assay. The assay is high-throughput adaptable and has a wide linear range from 10,000-400,000 cells. Therefore, in this study we used 30,000 of talcum powder and TiO_2 treated cells as well as control cells to stay within the recommended number of cells. The use of two layers of agar in the 96-well plate allowed the space to utilize such a high number of cells. The assay utilizes the conversion of tetrazolium salt to formazan by mitochondrial dehydrogenases which is directly proportional to the number of living transformed cells (Figure 2).

Tumor suppressor p53 gene mutations are frequently seen in ovarian cancers and can be used as a biomarker to differentiate low from high grade serous ovarian carcinomas. The methods used for the assessment of p53 (mutant vs. wild type) and Ki67 immunohistochemical expression in this study is identical to the methods used in clinical pathology laboratories for the diagnosis of the different subtypes of ovarian cancer. The slides were scored and interpreted independently by two pathologists. Mutant type p53 along with increased Ki67 expression were detected in both normal human primary ovarian epithelial (HPOE) and normal human ovarian epithelial cells (HOSEpiC) cells treated with 100 ug/ml talcum powder for 72 hours (Figure 4). These findings supported the malignant transformation of normal ovarian cells seen in the agar transformation assay.

The harmful biological effects of link between talcum powder exposure and ovarian cancer have been also confirmed in various in vitro cell culture studies [11, 22, 26-31, 38, 39]. Macrophage activation and inflammatory response to talcum powder were suggested as a link to increased risk of ovarian cancer [22, 27]. Macrophages exposed to nano-talc manifested increased levels in inflammatory markers, TNF-alpha, IL-1beta and IL-6 as well as

constituent phosphorylation of both p38 and ERK1/2 pathways [27]. p38 MAPK signaling pathway are known to be associated with cisplatin-resistant ovarian cancer [40]. Data suggest that nano-talc toxicity on human alveolar basal epithelial cells was mediated through oxidative stress [30]. Exposure of macrophages to talc and estradiol has led to increased production of reactive oxygen species and changes in expression of macrophage genes that play a role in cancer development and immunosurveillance [38]. These studies have also shown that ovarian cancer cells were present in larger numbers after co-culture with macrophages exposed to talc powder when in the presence of estradiol [38].

Oxidative stress and inflammation have been implicated in the pathogenesis of ovarian cancer, specifically, by increased expression of several key pro-oxidant enzymes in EOC tissues and cells as compared to normal cells indicating an enhanced redox state [41]. This redox state is further enhanced in chemoresistant EOC cells as evidenced by a further increase in key pro-oxidant enzymes and a decrease in anti-oxidant levels, suggesting a shift towards a pro-oxidant state [41]. Antioxidant enzymes, key regulators of cellular redox balance, are differentially expressed in various cancers, including ovarian [41].

Our laboratory was the first to confirm the cellular effect of talcum powder and provide a potential molecular mechanism [11]. Talcum powder exposure induced molecular changes in redox enzymes in normal ovarian cells similar to those known for ovarian cancer [4, 11]. In all talc-treated cells, there was a significant dose-dependent increase in key prooxidants with a concomitant decrease in key antioxidants enzymes. Remarkably, talcum powder exposure induced specific point mutations that are known to alter the activity in some of these key enzymes. The mechanism by which talcum powder alters the cellular redox and inflammatory

balance involves the induction of specific mutations in key oxidant and antioxidant enzymes that correlate with alterations in their activities. The fact that these mutations happen to correspond to known SNPs of these enzymes suggests a genetic predisposition to developing ovarian cancer with genital talcum powder use [11].

We have previously reported that EOC cells manifest increased cell proliferation and decreased apoptosis, a hallmark of malignant cells, as compared to normal ovarian epithelial cells [41]. Recently, we have shown that talcum powder further enhances cell proliferation and induces an inhibition in apoptosis in EOC cells, but more importantly in normal cells, suggesting talc is a stimulus to the development of the oncogenic phenotype [11]. Furthermore, CA-125, a membrane-bound and secreted protein, has been established as a biomarker for disease progression and response to ovarian cancer treatment [42]. CA-125 expression was significantly increased to values approaching clinical significance (35 U/ml in postmenopausal women) in talc treated human normal epithelial ovarian cells [11, 42]. Collectively, these findings confirmed the inflammatory/redox stress effects of talcum powder exposure to normal ovarian epithelial cells and indicated that this stress is a key mechanism in the malignant transformation of these cells.

The link between genital talcum powder use and ovarian cancer has been shown in numerous epidemiological studies. In addition, the inflammatory effects of talcum powder have been demonstrated in humans, animals, and cells in culture. This study which clearly demonstrates malignant transformation of normal ovarian cells in culture adds to the strong evidence of a causal relationship between the genital use of talcum powder and ovarian cancer.

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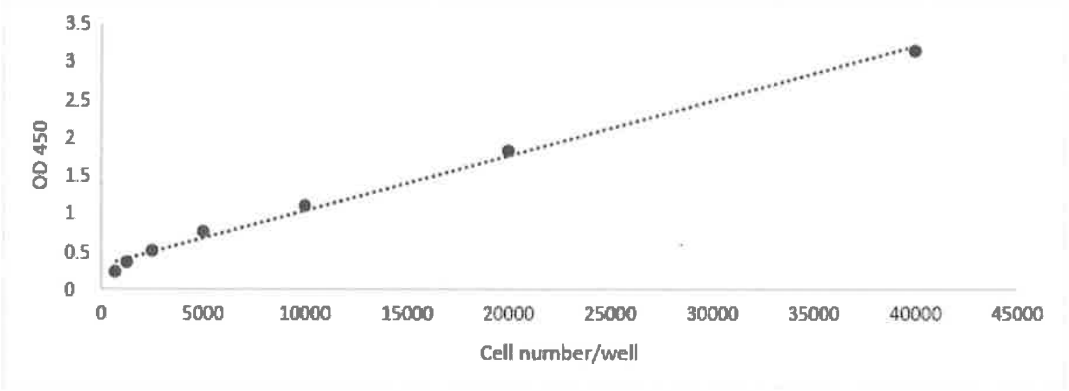

Figure 1: Human primary normal ovarian epithelial cell-dose curve. The cell dose curve was established as described in methods using a serial dilution of cells.

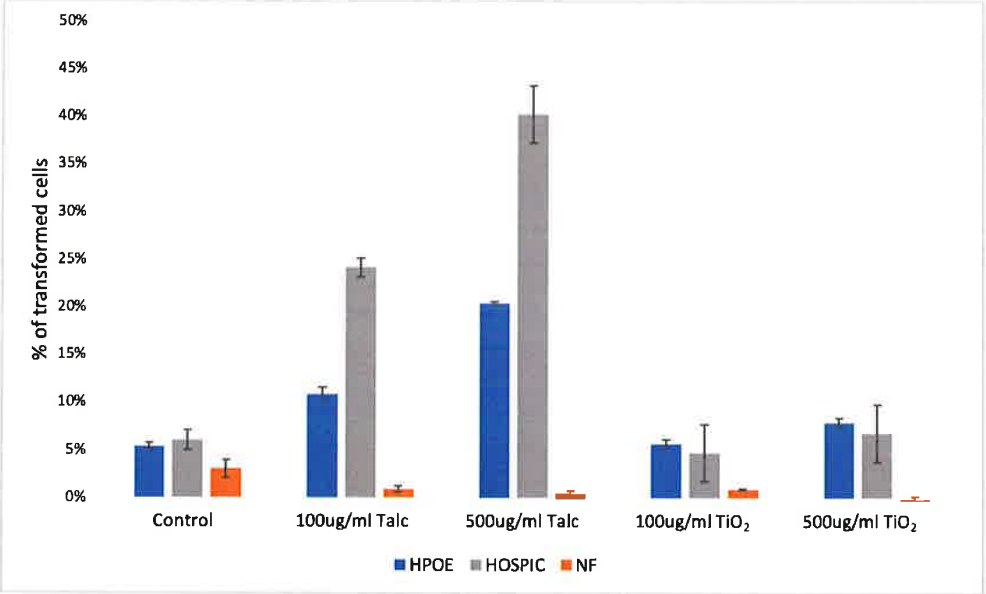

Figure 2: Equal numbers (30K) of human primary normal ovarian epithelial cells (HPOE), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) were seeded for the cell transformation assay as described in methods. After 6 days, the cell number were measured. Standard and samples readings were taken 4 hours after adding WST working solution. Control: cells (30K) with media only.

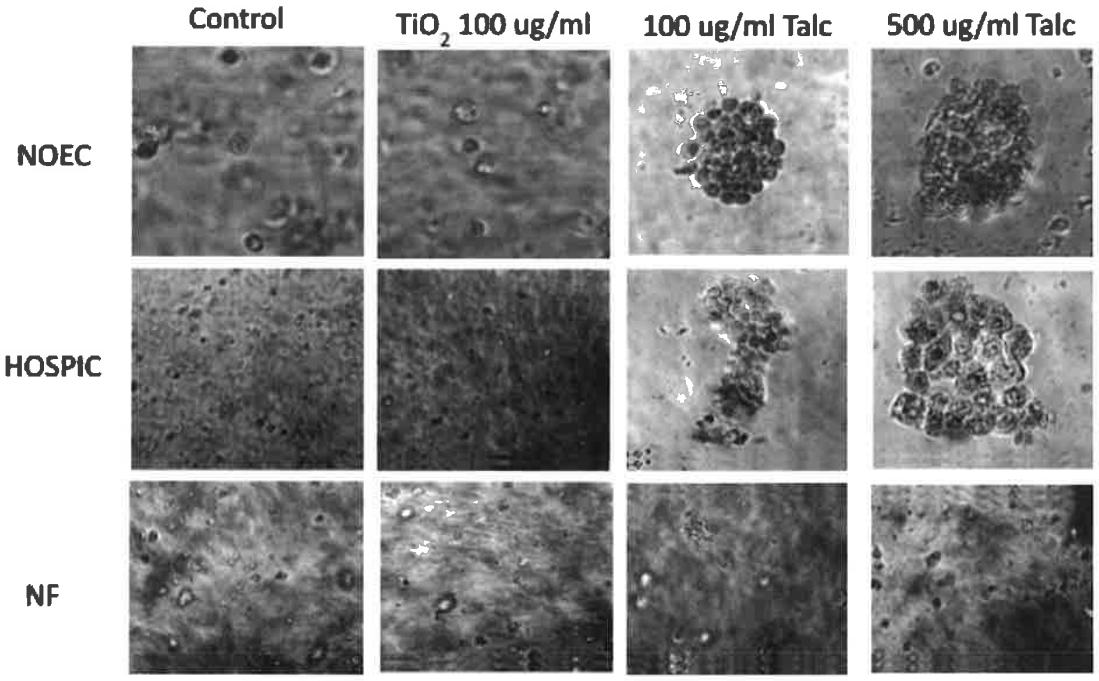

Figure 3: Images of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) treated with 100 and 500 ug/ml of talcum powder, after 6 days of culture. Colonies of transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase Contrast Microscope with an Axio camera.

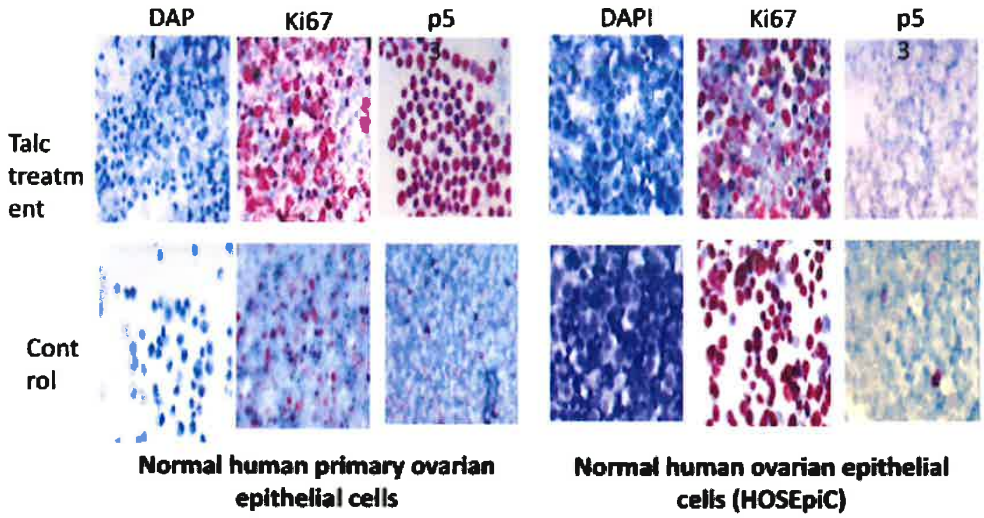

Figure 4: Immunohistochemistry staining for p53 and Ki67 in two normal human ovarian epithelial cells with and without Talcum powder (100 ug/ml) treatment for 72 hours. Slides were reviewed by two pathologists. Diffuse nuclear staining or complete negative staining with p53 is considered a positive reaction indicating mutated p53 status were observed in cells treated with talcum powder. Focal nuclear staining is consistent with wild type p53 and considered negative was observed in untreated cells (control). An increase in the proliferation index (Ki67) was observes in talcum powder treated cells versus controls.

Running title: Talcum powder use and risk of ovarian cancer

Title: Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells

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Key words: talc, talcum powder, titanium dioxide, epithelial ovarian cancer, transformation, primary cells, cell proliferation, colonies

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Declaration of Conflicting Interests: Dr. Saed has served as a paid consultant and expert witness for the plaintiffs in the talcum powder litigation. The remaining authors have no potential conflicts of interest to report.

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Author contributions:

Amy Harper: acquisition of data, study conception and design, critical revision

Xin Wang: acquisition of data

Rong Fan: acquisition of data

Nicole M. Fletcher: interpretation of data, study conception and design, critical revision

Robert T. Morris: interpretation of data, critical revision

Ghassan M. Saed: acquisition of data, study conception and design, drafting of manuscript, critical revision

Gynecologic Oncology
GYN-20-1870: Final Decision
February 2, 2021 at 12:27 AM
Ghassan M. Saed

[EXTERNAL]

Ms. No.: GYN-20-1870
Title: Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells
Corresponding Author: Dr. Ghassan M. Saed
Authors: Amy Harper, MD; Xin Wang, MS; rong fan, MS; Nicole Fletcher, Ph.D; Robert Morris, MD

Dear Dr. Saed,

Your paper, referenced above, has now been reviewed by at least two experts in the field and the Editors. Based on the reviewers' comments, we must inform you that while your work is not without merit, we are unable to accept your manuscript for publication in Gynecologic Oncology. In the last year we have seen a significant increase in the number of manuscripts submitted to the Journal and as a result, we are now accepting less than 20% of the manuscripts submitted to the Gynecologic Oncology.

We have attached the comments of the reviewers below in order for you to understand the basis for our decision. We hope that their thoughtful comments will help you in your future studies and possibly, with submission to another journal. Please note that a revised version of the current manuscript should not be submitted for another review to Gynecologic Oncology .

The critique of this paper in no way implies a lack of interest in this area of research, and we invite you to submit your future work to the Journal.

Sincerely,

Barbara A. Goff, MD
Editor
Gynecologic Oncology

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Reviewers' comments:

Reviewer #1: GYN-20-1870

Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells

This study examines the effects of talcum powder on normal human primary ovarian surface epithelium. The authors utilize an in vitro anchorage-independent growth assay to assess transformation of ovarian surface epithelial cells in response to talcum powder and follow this with immunohistochemical markers for proliferative index (Ki-67) and p53 expression patterns. The authors identified an increase in transformed cell percentage with increasing concentration of talcum powder, and correlated these findings with observed increases in Ki-67 proliferative index and abnormal p53 staining. The authors conclude that talcum powder induces malignant transformation of ovarian surface epithelial cells.

As presented, the manuscript presents several major issues that warrant attention prior to publication. Of primary concern is the reliance on a single commercial assay for assessment of transformation that has not been established in the literature. Moreover, appropriate statistical tests were not applied and thus the data are difficult to interpret. The clinical relevance is questionable given the arbitrary dose selection of talcum powder, and perhaps more importantly the examination of ovarian surface epithelial cells without comparison to fallopian tube secretory epithelium. Given that the prevailing evidence suggests the origin of high-grade serous ovarian cancer is the fallopian tube, the data presented are of limited relevance. Further demonstration of transformational changes is required before resubmission.

Specific Comments:

1. It is now generally accepted that most high-grade serous ovarian carcinomas arise from the fallopian tube (FT) fimbrial epithelium. Hence, these studies should examine the effects of talcum powder on FT cells. There are commercial (e.g., ATCC) and academic sources to obtain these cells.
2. Further, with the FT fimbria as the dominant site of origin for high-grade serous carcinoma, it is conceptually difficult

2. Further, with the FT fimbria as the dominant site of origin for high grade serous carcinoma, it is conceptually difficult to understand why ascending talcum powder would preferentially affect the fimbria and not the more proximal portions of the FT. The authors should address these concepts.
3. The reviewer recommends that further work be undertaken to establish whether talcum powder induces functional changes in ovarian epithelial cells suggestive of malignant transformation. The correlation to IHC is insufficient to draw this conclusion, and thus the results of this study are overinterpreted. Given that the transformed cells were not sub-cultured or further analyzed following treatment conditions to show phenotypic, genetic/epigenetic or functional changes, the changes seen at IHC may be explained by cellular responses to treatments. Whereas durable alterations in p53 staining may indicate mutations, as is the case clinically, p53 expression at a single time point following treatment cannot differentiate between novel mutations and physiologic responses to a given treatment condition. The IHC data would be further strengthened by functional data and/or genomic analysis.
4. Statistical analysis: Two major issues arise. The first is the use of paired t-test in an experiment with three treatment conditions (media, Talc, TiO2). This necessitates analysis of variance and multiple comparisons. Additionally, significance in not denoted in figure 2. The authors conclude that proliferative index is elevated to 90%, but do not show evidence or statistical analysis to this effect. Moreover, the methods describe a binary method of scoring Ki-67 of high or low, calling into question how the 90% value was generated.
5. Figure 1: While the standard curve establishes the ability of the assay to detect cell number, it offers little to the reading of the manuscript and could be reserved for supplementary data.
6. The difference between HPOE and HOSEpiC needs to be mentioned. Are HOSEpiC immortalized in some way?
7. Figure 2: Statistical significance is not displayed on the figure, nor are important statistical comparisons between the no treatment control and TiO2
8. Figure 3: NOEC 100ug/mL and HOSPIC 500ug/ML Talc panels appear to be identical. TiO2 at 500ug/mL is conspicuously missing.
9. Figure 4: Top left panel (DAP/Talc) is identical to DAP/Control. Inadequate white balancing detracts from the reader's ability to appreciate colorimetric differences between the panels.
10. Several phrases describing cell culture and colorimetric assay within the methods and discussion appear to be taken verbatim from the manufacturers' websites: examples below.

o Line 80-82: Cells were grown in T25 tissue culture flasks precoated with gelatin-based coating solution for 2 min and incubated in Cell Biologics' Culture Complete Growth Medium which generally took 3-7 days.

From Cell Biologics website: "Human Primary Ovarian Epithelial Cells are grown in T25 tissue culture flasks pre-coated with gelatin-based coating solution for 2 min and incubated in Cell Biologics' Culture Complete Growth Medium generally for 3-7 days."

o Line 126-130: "This assay was chosen because it is more stable, faster and more sensitive than the traditional Soft-Agar Assay that is lengthy (3-4 weeks incubation), laborious (counting colonies) and inconsistent (due to subjective counting). The assay is high-throughput adaptable and has a wide linear range from 10,000-400,000 cells."

From abcam website: "…is lengthy (3-4 weeks incubation), laborious (counting colonies) and inconsistent (due to subjective counting)… The assay is high-throughput adaptable and has a wide linear range from 10,000-400,000 cells."

o Line 185: "Anchorage-independent growth is a hallmark of cancer cells"

From abcam website: "Anchorage-independent cell growth is the hallmark of cell transformation"

o Line 223-226: The kit is based on the conversion of the tetrazolium salt (WST) to formazan by cellular mitochondrial dehydrogenases. The generated signal is directly proportional to the number of living cells and thus can accurately determine number of transformed cells.

From abcam website: The kit is based on the conversion of the tetrazolium salt (WST) to formazan by cellular mitochondrial dehydrogenases. The generated signal is directly proportional to the number of living cells.

Reviewer #2: Ultimately these data are too premature for publication, the authors present very preliminary in vitro data suggesting that talcum powder may induce malignant change in normal ovarian epithelial cells, but not in fibroblasts. The data are premature, restricted to two cell lines and really offer no significant mechanistic insight.
I also think the dose of talcum powder is extremely high, I calculate it to be 263mM for the lower dose which is unlikely to ever replicate physological dosing and although the authors recognise this in the discussion it is a major experimental flaw and makes interpretation of results very difficult. The use of IHC to determine p53 mutation status is not very senstiive and I would suggest this needs to be confirmed with sequencing

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January 04, 2021

To: Editor-in-Chief, *Gynecologic Oncology*

Please consider our priority report entitled ***Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells*** for publication in *Gynecologic Oncology*. In this study, we determined that exposure to talcum powder induces malignant transformation in human normal ovarian epithelial cells but not in human normal peritoneal fibroblasts. These findings represent a direct effect of talcum powder exposure that is specific to normal ovarian cells and further supports previous studies demonstrating an association between the genital use of talcum powder and an increased risk of ovarian cancer. This study is fitting for *Gynecologic Oncology* because of the potential ovarian cancer risk that is associated with talcum powder use. The material contained in the manuscript has not been published, has not been submitted, or is not being submitted elsewhere for publication.

Author contributions:
Amy Harper: acquisition of data, study conception and design, critical revision
Xin Wang: acquisition of data
Rong Fan: acquisition of data
Nicole M. Fletcher: acquisition of data, interpretation of data, study conception and design, critical revision
Robert T. Morris: interpretation of data, critical revision
Ghassan M. Saed: study conception and design, drafting of manuscript, critical revision

Sincerely,

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Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells

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Key words: talc, talcum powder, titanium dioxide, epithelial ovarian cancer, transformation, primary cells, cell proliferation, colonies

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Abstract

Objective: Several studies have linked perineal use of talcum powder to increased risk of ovarian cancer (OC). Here, we determined that exposure to talcum powder induces malignant transformation in human normal ovarian cells.

Methods: Human primary ovarian epithelial cells (HPOE), ovarian epithelial cells (HOSEpiC), and primary fibroblasts (NF) were treated with either 100 or 500 ug/ml of talcum powder or titanium dioxide (TiO_2) as a particulate control for 72 hours before assessment with a cell transformation assay and p53 and Ki67 immunohistochemistry.

Results: Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in a dose dependent manner in ovarian cell lines. No colonies formed in the untreated ovarian cells or control ovarian cells (TiO_2 treated) at either dose. There were no colonies formed in talc treated NF cells. Transformed ovarian cells were increased by 11% and 20% in HPOE and 24% and 40% in HOSEpic cells for talcum powder 100 and 500 ug/ml doses, respectively ($p<0.05$). There were no detectible transformed cells when cells were treated with TiO_2. Importantly, p53 mutant type as well as increased expression of Ki67 were detected in HPOE and HOSEpic cells when exposed to talcum powder.

Conclusion: Exposure to talcum powder induces malignant transformation in ovarian epithelial cells but not in NF cells. These findings represent a direct effect of talcum powder exposure that is specific to normal ovarian cells and further supports previous studies demonstrating an association between the genital use of talcum powder and an increased risk of OC.

Introduction

Ovarian cancer is a gynecologic malignancy that ranks fifth in cancer deaths among women in United States [1]. Epithelial ovarian cancer (EOC) presents with various histopathology, molecular biology, and clinical outcome and is therefore considered a heterogeneous disease [2]. The prognosis of EOC remains poor, with a 5-year survival rate of 50% in advanced stage [3]. This is largely due to the lack of early warning symptoms, screening methods, and the eventual development of chemoresistance [3].

The pathogenesis of EOC is strongly associated with oxidative stress and inflammation [4-8]. Epithelial ovarian cancer cells manifest a persistent pro-oxidant state that has been demonstrated in vitro and is also enhanced in chemoresistant EOC cells [4, 6]. Attenuation of the pro-oxidant state with antioxidants/scavengers has been shown in vitro to selectively induce apoptosis in EOC cells indicating a potential therapeutic value [5, 9, 10]. Talcum Powder has also been shown to induce oxidative stress and cell proliferation and to decrease apoptosis in ovarian cancer cells and in normal ovarian cells [11].

The association between genital use of talcum powder and risk of ovarian cancer have been described in numerous studies [11-18]. Several meta-analyses have demonstrated a statistically significant increased risk of ovarian cancer with the genital use of talcum powder [16, 19, 20]. In addition, several animal studies have reported that talcum powder causes inflammation and oxidative stress [21-25]. Several *in vitro* studies have demonstrated a biologic effect when cells in culture are exposed to talcum powder [26-31]. In support of these previous findings, we have recently delineated the molecular basis of the association of talcum powder use with increased risk of ovarian cancer [11].

Despite these concerns, the specific mechanism by which talcum powder exposure causes ovarian cancer has not been definitively established.

Here we clearly demonstrate that exposure to talcum powder induces malignant transformation in human primary normal ovarian epithelial cells and thus, providing a mechanism for the increased risk of ovarian cancer with the genital use of talcum powder.

Material and Methods

Cell lines:

Normal human primary ovarian epithelial cells (HPOE): Cells were purchased from Cell Biologics, Chicago, IL. Cells were received at passage 3 cryo-preserved in vials containing at least $0.5x10^6$ cells per ml. Cells were grown in T25 tissue culture flasks pre-coated with gelatin-based coating solution for 2 min and incubated in *Cell Biologics'* Culture Complete Growth Medium which generally took 3-7 days. Cells were expanded for 2-4 passages at a split ratio of 1:2 under the cell culture conditions as specified by *Cell Biologics.* Human Epithelial Cell Medium is a complete medium designed for the culture of human epithelial cells. It was tested and optimized with epithelial cell growth and proliferation in vitro. Cells were incubated at 37 °C with 5% CO_2 and 95% air in a humidified incubator. The medium consists of 500 ml of basal medium (containing essential and non-essential amino acids, vitamins, organic and inorganic compounds, hormones, growth factors, trace minerals), supplemented with epithelial cell growth supplement, antibiotics, and fetal bovine serum.

Normal human ovarian epithelial cells (HOSEpiC): Cells were purchased from ScienCell Research Laboratories, Inc, Carlsbad, California. HOSEpiC cells were isolated

from human ovary. HOSEpiC cells were received cryopreserved at passage one in frozen vials, each vial contains >5 x 10^5 cells in 1 ml volume. HOSEpiC were further expanded for 2-3 passages under the conditions provided by ScienCell Research Laboratories. Cells were grown in Ovarian Epithelial Cell Medium (OEpiCM, Cat. #7311). Cells were incubated at 37°C with 5% CO_2 and 95% air in a humidified incubator.

Human normal primary peritoneal fibroblasts: Cells were isolated and cultured as we have previously described [32, 33]. The fibroblast cell line has been extensively characterized in previous studies and has been shown to be pure and solely fibroblast cells [32, 33]. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, CA), supplemented with 10 % fetal bovine serum (FBS, Innovative Research, Novi, MI) and penicillin/streptomycin (Fisher Scientific, Waltham, MA) as we have previously described [32, 33]. Cells were incubated at 37 °C with 5% CO_2 and 95% air in a humidified incubator.

Talcum powder treatment: Talcum baby powder (Johnson & Johnson, New Brunswick, NJ, #30027477, Lot#13717RA) or control particles, Titanium dioxide (TiO_2, Spectrum Chemical Corp, Lot No. 2EB0148) were used to treat cells. Talcum powder or TiO_2 were suspended in PBS (Stock solution of 50 mg/ml) and sonicated 3 times for 1 minute each with Sonic Dismembrator (Fischer Scientific, Model 100). Stock solutions were filtered through 30 µm nylon mesh filters. No visible loss of material has observed. Cells were seeded in 100 mm Petri cell culture dishes (1 x 10^6) and were treated 24 hours later in duplicate in a fresh media with 100 or 500 µg/ml of talc or titanium dioxide (TiO_2) for 72 hours. Control: cells (30K) with media only and Negative control: cells (30K) with media and PBS. No cell death was observed after 72 hours in culture in control or treated cells.

Titanium dioxide, a naturally occurring particle, has been classified in humans and animals as biologically inert [28, 34]. Titanium dioxide particles are produced and used as fine (~ 0.1-2.5 µm) and nanosize (<0.1 µm) particles [34]. In this study, we used TiO_2 as a particulate control to exclude the effect of material size. Culture plates were washed several times to remove residual particles and collected by trypsin in fresh media. Cells were counted and their concentration was adjusted with fresh media to 1.5 X 10^6 cells/ml.

Cells were now ready to be assessed with cell transformation assay (colorimetric), according to the manufacturer protocol (Abcam-235698, Cambridge, MA). The 100 and 500 ug/ml doses were chosen based on our previous studies which showed talcum powder to induce changes in redox balance of cells at the molecular level [11]. The experiments were repeated 3 times with a fresh solution of talcum powder and TiO_2. This assay was chosen because it is more stable, faster and more sensitive than the traditional Soft-Agar Assay that is lengthy (3-4 weeks incubation), laborious (counting colonies) and inconsistent (due to subjective counting). The assay is high-throughput adaptable and has a wide linear range from 10,000-400,000 cells.

A Cell-dose curve was established as described in the manufacturer's protocol. Briefly, we used cells (5.34 x 10^5 cells/ml) were suspended in 1X DMEM/10% FBS medium. Cells were diluted into seven serial dilutions in a 1.5 mL centrifuge tubes. Serial dilution was performed using an 8 channel multi pipette by adding 150 µl of media to each well of a 96 well microplate. A 150 µl aliquot of the 5.34 x10^5 cells/ml (80 x10^3 cells) was added to the wells of the first duplicate row. A 150 µl aliquot from the first duplicate row was removed and added to the next well and mixed. The process was repeated until the seven serial dilutions were obtained. The final well was blank with media only and no

cells. A 35 µl aliquot of 1X DMEM/10% FBS and 15 µl of WST working solution were then added into each well and incubate at 37° C for 4 hours. The absorbance was measured by a microtiter plate reader at 450 nm (Figure 1).

Agarose and WST working solutions were prepared as described in the kit information sheet (Abcam-235698, Cambridge, MA). The base agarose mix was added into the required wells in a 96 well plate and kept for 15 minutes at 4°C to solidify the agarose. A top agarose layer stock solution was prepared by using talcum powder or TiO_2 treated stock cell solution of 1.5 X 10^6 cells/ml (30,000 cells per well, which is within the recommended range of the assay) in 1X DMEM/10% FBS medium. The agarose-cell mix was added into every well of a 96 well plate previously holding the solidified base agarose layer and placed at 4°C for 10 minutes to solidify the layer. After placing the plate for 10 min at 37°C, 1X DMEM/10% FBS medium was added to all the wells and incubated at 37°C for 6-8 days. On the last day the upper medium on the top agarose layer was cautiously removed by pipetting. A 1X DMEM/10% FBS and WST working solution was added into each well, incubated for 4 hours at 37°C. The absorbance was measured by a microtiter plate reader at 450 nm. Colonies of transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase Contrast Microscope with an Axio camera.

Immunohistochemistry (IHC) staining and scoring: The IHC panel consisted of antibodies against p53 and Ki-67. The primary antibodies, suppliers, and staining conditions are listed below.

Antibody	Clone	Source	Detection System	Dilution
P53	DO-7	Ventana	Ventana ultraView DAB	1:500
Ki-67	Mib1	Ventana	Ventana ultraView DAB	1:2000

Cytospin slides were prepared from cells and stained using immunoperoxidase labeling performed with the automated XT iVIEW DAB V.1 procedure on the Ultra BenchMark XT IHC/ISH Staining Module, Ventana with anti-p53 (clone DO-7 prediluted, Ventana). Antigen retrieval was carried out with CC1, pH 8.0 (Ventana). Sections were incubated with primary antibodies for 36 min at 37°C. All slides were reviewed by two pathologists (Drs Ali and Alrajjal). Cases with discordant Ki-67 estimated results underwent a consensus review at a double-headed microscope. Diffuse "in-block" nuclear staining or complete negative staining with p53 was considered a positive reaction indicating mutated p53 status. Focal nuclear staining is consistent with "wild type" p53 and considered negative. The Proliferation Index (PI) was assessed qualitatively using Ki-67-stained slides and classified as high PI (>50% positive cells) or low PI (<50% positive cell).

Statistical Analysis: Data was analyzed with paired t-tests. Data are expressed, as means +/- SD. Significance was determined as $P < 0.05$.

Results

Treatment with talcum powder significantly increased the number of transformed normal epithelial ovarian cells by 11% and 20% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, $p<0.05$). Likewise, but to a greater extent, treatment with talcum powder significantly increased the number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, $p<0.05$). Talcum powder had no detectable transformation effect on normal peritoneal fibroblasts (Figure 2).

Anchorage-independent growth is a hallmark of cancer cells. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in normal epithelial ovarian cell lines in a dose dependent manner (Figure 3). There were no colonies formed in talcum powder treated normal fibroblasts (Figure 3). There were no colonies formed in either untreated ovarian cells or control ovarian cells at either dose. There were no detectible transformed cells when cells were treated with the particulate control, TiO_2.

To confirm malignant cell transformation observed with the cell transformation assay used in this study we performed IHC on the normal human primary ovarian epithelial (HPOE) and normal human ovarian epithelial cells (HOSEpiC) cells staining for p53 and Ki67. Focal p53 nuclear staining indicating wild type p53 expression was observed in cells before treatment. After treatment of cells with talcum powder 100 ug/ml for 72 hours, diffused "in-block" nuclear staining was observed indicating p53 mutated form (Figure 4). Additionally, talcum powder treatment increased the proliferation index (PI) in both cell lines. The baseline PI for HPOE and HOSEpic cells was 50 and 70% respectively. The PI was significantly increased to 90% in both cell lines (Figure 4).

Discussion

This is the first study to directly show that exposure to talcum powder induces transformation in normal human ovarian epithelial cells. The ability of talcum powder exposure to induce transformation appears to be specific to ovarian cells as it did not induce transformation in human normal peritoneal fibroblasts. (Figure 3). The reason for this specific effect of talcum powder on the ovaries in still under investigation.

The dose and time of talcum powder exposure in cell culture experiments used in this study was based on previous studies [11]. These doses are not intended to represent a typical dose when applied to the genital area in women over time. Despite this limitation, the development and use of in vitro models has been valuable in the advancement of research and knowledge on cancer pathogenesis [35]. The cellular transformation demonstrated in this study was significant and informative.

The soft agar in vitro colony formation assay is widely accepted and used to evaluate cellular transformation *[36, 37]* . Anchorage-independent growth is one of the hallmarks of cell transformation and is accepted to be the most accurate and stringent in vitro assay for detecting malignant transformation of cells [36, 37]. In this study we used Cell Transformation Assay Kit, which is faster, stable, and more sensitive than the traditional Soft-Agar Assay. The assay is high-throughput adaptable and has a wide linear range from 10,000-400,000 cells. Therefore, in this study we used 30,000 of talcum powder and TiO_2 treated cells as well as control cells to stay within the recommended number of cells. The use of two layers of agar in the 96-well plate allowed the space to utilize such a high number of cells. The kit is based on the conversion of the tetrazolium salt (WST) to formazan by cellular mitochondrial dehydrogenases. The generated signal is directly proportional to the number of living cells and thus can accurately determine number of transformed cells (Figure 2).

Tumor suppressor p53 gene mutations are frequently seen in ovarian cancers and can be used as a biomarker to differentiate low from high grade serous ovarian carcinomas. The methods used for the assessment of p53 (mutant vs. wild type) and Ki67 immunohistochemical expression in this study is identical to the methods used in clinical

pathology laboratories for the diagnosis of the different subtypes of ovarian cancer. The slides were scored and interpreted independently by two pathologists. Mutant type p53 along with increased Ki67 expression were detected in both normal human primary ovarian epithelial (HPOE) and normal human ovarian epithelial cells (HOSEpiC) cells treated with 100 ug/ml talcum powder for 72 hours (Figure 4). These findings supported the malignant transformation of normal ovarian cells seen in the agar transformation assay.

The harmful biological effects of link between talcum powder exposure and ovarian cancer have been also confirmed in various in vitro cell culture studies [11, 22, 26-31, 38, 39]. Macrophage activation and inflammatory response to talcum powder were suggested as a link to increased risk of ovarian cancer [22, 27]. Macrophages exposed to nano-talc manifested increased levels in inflammatory markers, TNF-alpha, IL-1beta and IL-6 as well as constituent phosphorylation of both p38 and ERK1/2 pathways [27]. p38 MAPK signaling pathway are known to be associated with cisplatin-resistant ovarian cancer [40]. Data suggest that nano-talc toxicity on human alveolar basal epithelial cells was mediated through oxidative stress [30]. Exposure of macrophages to talc and estradiol has led to increased production of reactive oxygen species and changes in expression of macrophage genes that play a role in cancer development and immunosurveillance [38]. These studies have also shown that ovarian cancer cells were present in larger numbers after co-culture with macrophages exposed to talc powder when in the presence of estradiol [38].

Oxidative stress and inflammation have been implicated in the pathogenesis of ovarian cancer, specifically, by increased expression of several key pro-oxidant enzymes

in EOC tissues and cells as compared to normal cells indicating an enhanced redox state [41]. This redox state is further enhanced in chemoresistant EOC cells as evidenced by a further increase in key pro-oxidant enzymes and a decrease in anti-oxidant levels, suggesting a shift towards a pro-oxidant state [41]. Antioxidant enzymes, key regulators of cellular redox balance, are differentially expressed in various cancers, including ovarian [41].

Our laboratory was the first to confirm the cellular effect of talcum powder and provide a potential molecular mechanism [11]. Talcum powder exposure induced molecular changes in redox enzymes in normal ovarian cells similar to those known for ovarian cancer [4, 11]. In all talc-treated cells, there was a significant dose-dependent increase in key prooxidants with a concomitant decrease in key antioxidants enzymes. Remarkably, talcum powder exposure induced specific point mutations that are known to alter the activity in some of these key enzymes. The mechanism by which talcum powder alters the cellular redox and inflammatory balance involves the induction of specific mutations in key oxidant and antioxidant enzymes that correlate with alterations in their activities. The fact that these mutations happen to correspond to known SNPs of these enzymes suggests a genetic predisposition to developing ovarian cancer with genital talcum powder use [11].

We have previously reported that EOC cells manifest increased cell proliferation and decreased apoptosis, a hallmark of malignant cells, as compared to normal ovarian epithelial cells [41]. Recently, we have shown that talcum powder further enhances cell proliferation and induces an inhibition in apoptosis in EOC cells, but more importantly in normal cells, suggesting talc is a stimulus to the development of the oncogenic phenotype

[11]. Furthermore, CA-125, a membrane-bound and secreted protein, has been established as a biomarker for disease progression and response to ovarian cancer treatment [42]. CA-125 expression was significantly increased to values approaching clinical significance (35 U/ml in postmenopausal women) in talc treated human normal epithelial ovarian cells [11, 42]. Collectively, these findings confirmed the inflammatory/redox stress effects of talcum powder exposure to normal ovarian epithelial cells and indicated that this stress is a key mechanism in the malignant transformation of these cells.

The link between genital talcum powder use and ovarian cancer has been shown in numerous epidemiological studies. In addition, the inflammatory effects of talcum powder have been demonstrated in humans, animals, and cells in culture. This study which clearly demonstrates malignant transformation of normal ovarian cells in culture adds to the strong evidence of a causal relationship between the genital use of talcum powder and ovarian cancer.

Acknowledgment: The authors would like to acknowledge Dr. Ruba Ali-Fahmi and Dr. Ahmad Alrajjal from the department of pathology at Wayne State University who helped with immunohistochemistry of p53 and Ki67.

Declaration of Conflicting Interests: Dr. Saed has served as a paid consultant and expert witness for the plaintiffs in the talcum powder litigation. The remaining authors have no potential conflicts of interest to report.

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Author contributions:

Amy Harper: acquisition of data, study conception and design, critical revision

Xin Wang: acquisition of data

Rong Fan: acquisition of data

Nicole M. Fletcher: interpretation of data, study conception and design, critical revision

Robert T. Morris: interpretation of data, critical revision

Ghassan M. Saed: acquisition of data, study conception and design, drafting of manuscript, critical revision

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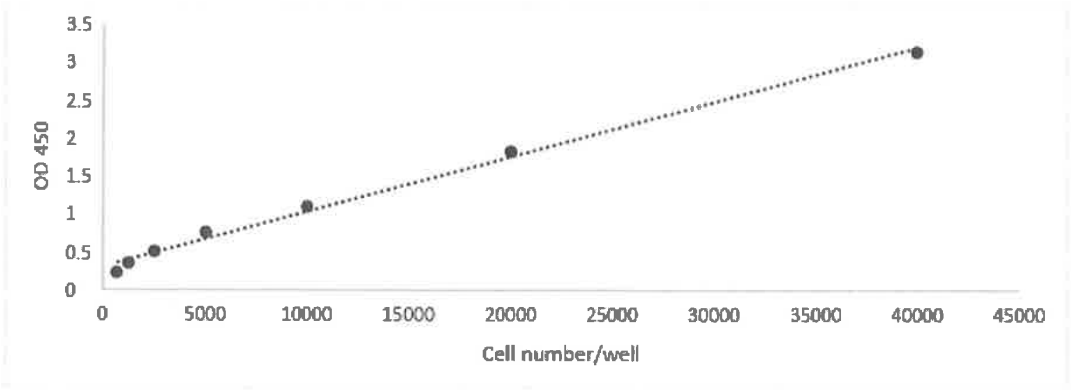

Figure 1: Human primary normal ovarian epithelial cell-dose curve. The cell dose curve was established as described in methods using a serial dilution of cells.

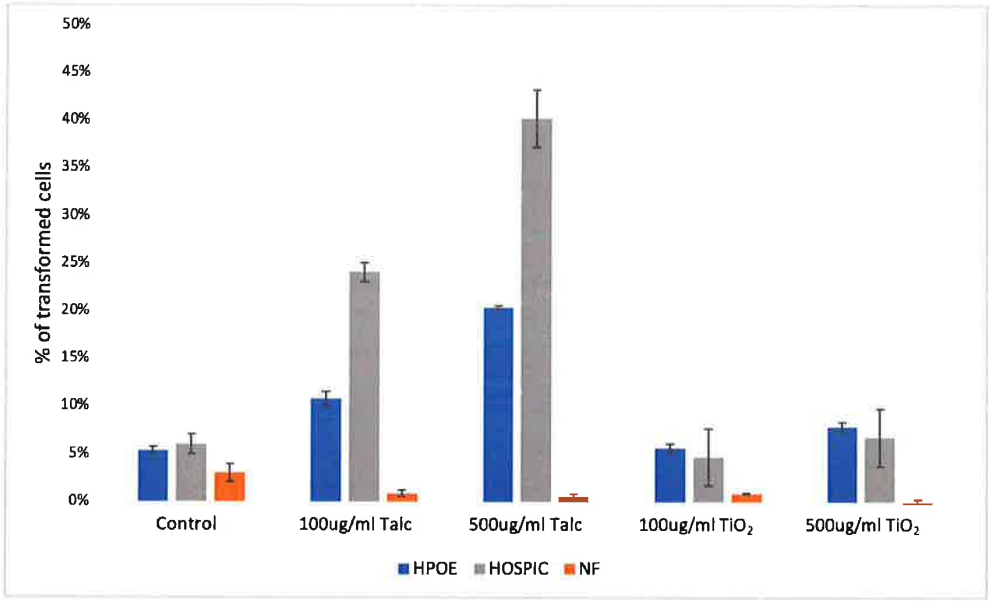

Figure 2: Equal numbers (30K) of human primary normal ovarian epithelial cells (HPOE), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) were seeded for the cell transformation assay as described in methods. After 6 days, the cell number were measured. Standard and samples readings were taken 4 hours after adding WST working solution. Control: cells (30K) with media only.

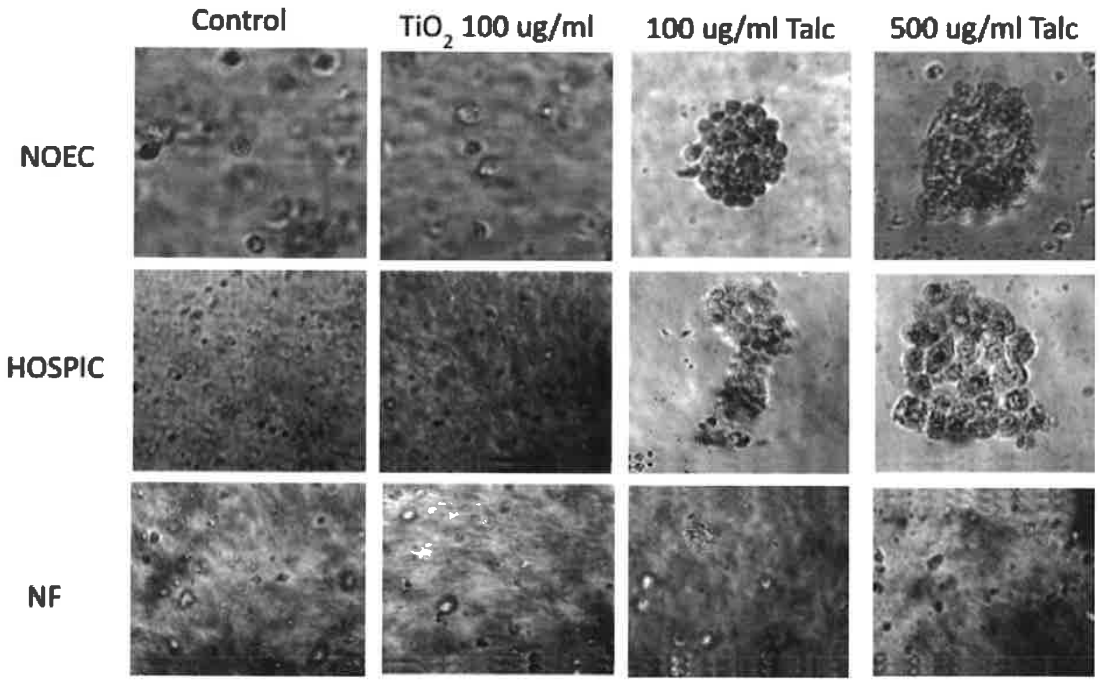

Figure 3: Images of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) treated with 100 and 500 ug/ml of talcum powder, after 6 days of culture. Colonies of transformed cells were detected and photograph by a Zeiss Axiovert 40 C Inverted Phase Contrast Microscope with an Axio camera.

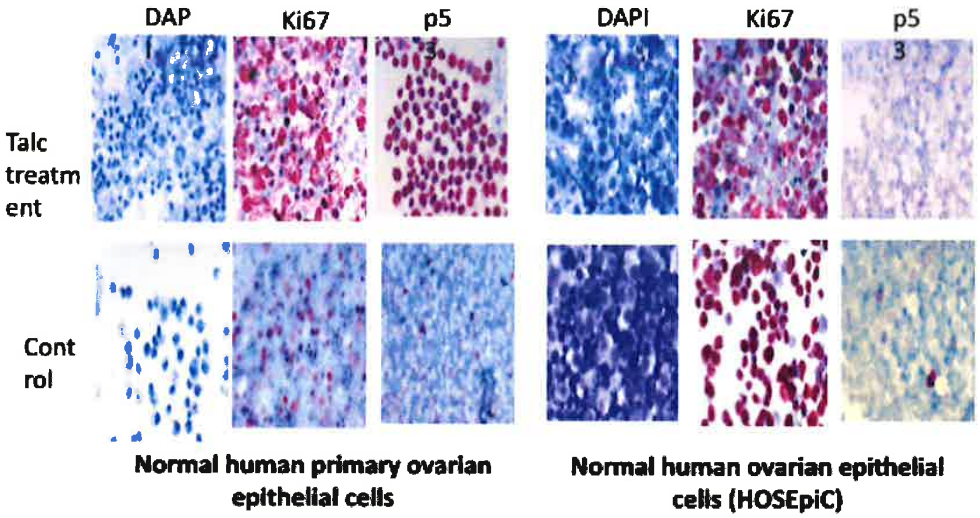

Figure 4: Immunohistochemistry staining for p53 and Ki67 in two normal human ovarian epithelial cells with and without Talcum powder (100 ug/ml) treatment for 72 hours. Slides were reviewed by two pathologists. Diffuse nuclear staining or complete negative staining with p53 is considered a positive reaction indicating mutated p53 status were observed in cells treated with talcum powder. Focal nuclear staining is consistent with wild type p53 and considered negative was observed in untreated cells (control). An increase in the proliferation index (Ki67) was observes in talcum powder treated cells versus controls.

Highlights

- Exposure to talcum powder induces malignant transformation in ovarian epithelial cells
- Exposure to talcum powder did not induce malignant transformation in primary normal fibroblasts
- Mutant p53 type and increased expression of Ki67 were detected in ovarian cells when exposed to talcum powder

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Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in six parts.

1. Identifying information.

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This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes".

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This section asks about patents and copyrights, whether pending, issued, licensed and/or receiving royalties.

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Definitions.

Entity: government agency, foundation, commercial sponsor, academic institution, etc.
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Section 1. Identifying Information

1. Given Name (First Name): Ghassan
2. Surname (Last Name): Saed
3. Date: 04-January-2021

4. Are you the corresponding author? ☑ Yes ☐ No

5. Manuscript Title: Talcum powder induces malignant transformation in normal human primary ovarian epithelial cells

6. Manuscript Identifying Number (if you know it):

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Did you or your institution **at any time** receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Are there any relevant conflicts of interest? ☑ Yes ☐ No

If yes, please fill out the appropriate information below. If you have more than one entity press the "ADD" button to add a row. Excess rows can be removed by pressing the "X" button.

Name of Institution/Company	Grant?	Personal Fees?	Non-Financial Support?	Other?	Comments
Beasly Allen Law Firm	☐	☑	☐	☐	A portion of Dr. Saed's time conducting this research was paid for by lawyers representing plaintiffs in the talcum powder litigation. Dr. Saed received no financial support for the authorship or publication of this article.

Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were **present during the 36 months prior to publication.**

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Name of Entity	Grant?	Personal Fees?	Non-Financial Support?	Other?	Comments
Beasly Allen Law Firm	☐	☑	☐	☑	Served as a paid consultant and expert witness for the plaintiffs in the talcum powder litigation

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At the time of manuscript acceptance, journals will ask authors to confirm and, if necessary, update their disclosure statements. On occasion, journals may ask authors to disclose further information about reported relationships.

Section 6. Disclosure Statement

Based on the above disclosures, this form will automatically generate a disclosure statement, which will appear in the box below.

Dr. Saed reports personal fees from Beasly Allen Law Firm, during the conduct of the study; personal fees and other from Beasly Allen Law Firm, outside the submitted work; .

Evaluation and Feedback

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Subject: PLOS ONE Decision: PONE-D-20-29874 - [EMID:d5132363bdcb6a7c]
Date: October 28, 2020 at 9:17 AM
To: Ghassan M Saed gsaed@med.wayne.edu

PO

[EXTERNAL]

PONE-D-20-29874
Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells
PLOS ONE

Dear Dr. Saed,

Thank you for submitting your manuscript to PLOS ONE. After careful consideration, we have decided that your manuscript does not meet our criteria for publication and must therefore be rejected.

Specifically:
==========

Both reviewers have raised serious concerns about the experimental design, analyses and interpretation of the findings.

==========

I am sorry that we cannot be more positive on this occasion, but hope that you appreciate the reasons for this decision.

Yours sincerely,

Salik Hussain, D.V.M, M.S., Ph.D.,
Academic Editor
PLOS ONE

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Reviewers' comments:

Reviewer's Responses to Questions

Comments to the Author

1. Is the manuscript technically sound, and do the data support the conclusions?

The manuscript must describe a technically sound piece of scientific research with data that supports the conclusions. Experiments must have been conducted rigorously, with appropriate controls, replication, and sample sizes. The conclusions must be drawn appropriately based on the data presented.

Reviewer #1: No

Reviewer #2: No

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2. Has the statistical analysis been performed appropriately and rigorously?

Reviewer #1: No

Reviewer #2: No

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3. Have the authors made all data underlying the findings in their manuscript fully available?

The PLOS Data policy requires authors to make all data underlying the findings described in their manuscript fully available without restriction, with rare exception (please refer to the Data Availability Statement in the manuscript PDF file). The data should be provided as part of the manuscript or its supporting information, or deposited to a public repository. For example, in addition to summary statistics, the data points behind means, medians and variance measures should be available. If there are restrictions on publicly sharing data—e.g. participant privacy or use of data from a third party—those must be specified.

Reviewer #1: Yes

Reviewer #2: Yes

4. Is the manuscript presented in an intelligible fashion and written in standard English?

PLOS ONE does not copyedit accepted manuscripts, so the language in submitted articles must be clear, correct, and unambiguous. Any typographical or grammatical errors should be corrected at revision, so please note any specific errors here.

Reviewer #1: Yes

Reviewer #2: No

5. Review Comments to the Author

Please use the space provided to explain your answers to the questions above. You may also include additional comments for the author, including concerns about dual publication, research ethics, or publication ethics. (Please upload your review as an attachment if it exceeds 20,000 characters)

Reviewer #1: General Comments
The manuscript by Harper et al. entitled 'Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells' describes an in vitro study assessing talc powder ability to induce malignant transformation in human ovarian cell lines. The authors conducted a 72 hour exposure of talc powder or titanium dioxide to separate cultures of primary ovarian epithelial, ovarian epithelial, or primary peritoneal fibroblast cells. This was followed by plating exposed cells into agar-based cell transformation assay based on formazan dye absorbance measurement. The authors report a dose-dependent increase in transformed ovarian epithelial cells for only talc powder, and conclude that talc powder exposure causes malignant transformation in these cells. Although this study's subject is timely and warranted based on the current literature, several critical fatal flaws in the study included no description of talc powder and TiO2 characteristics, seeding density in the soft agar assay, and use of a single in vitro soft agar assay to claim malignant transformation. Critiques and suggestions for improvement are given as follows:

Major Comments
1) No hypothesis was stated in either the Abstract or the Introduction section. Please provide.
2) It was not clear or explained how an acute 72 hour exposure to talc powder leads to cell transformation. This suggests that a single application of talc is extremely potent and carries high risk for cell transformation. Since talc powder is widely used, why aren't cancer rates much, much higher? For most carcinogens, repeated exposures over long periods of time drive carcinogenesis. Without adequate discussion or further data to support this claim, this finding is highly questionable.
3) In the abstract the authors state 'This finding represents a direct causation mechanism of talcum powder exposure.' Colony formation does not show molecular mechanism of action. At best, it describes the end product of a mechanism and a mode of action. Please revise.
4) Based on the minimal amount of data provided in this manuscript, the authors' conclusions suggesting acute exposure of talc powder to ovary epithelial cells is associated with ovarian cancer are outrageous and not supported by the manuscript's data.
5) The authors state their objective was 'to determine whether exposure to talcum powder will induce malignant transformation.' The in vitro method used did not address this objective. Soft agar colony formation alone in an in vitro test system is not enough data to claim malignant transformation. At best, the authors should claim 'cell transformation'. Malignant means cancer, while neoplasm refers cells that show tumor-like properties. To show 'neoplastic transformation', authors would need to conduct a more diverse battery of tests to show that these 'transformed' cells possess a tumor or cancer cell phenotype (i.e. cancer hallmarks), as outlined by Hannahan and Weinberg. Furthermore, the authors would need to show that the tested talc powder possesses a majority of the key characteristics that define a carcinogen (i.e. Smith et al. 2016), which is the set of criteria that IARC now uses in its carcinogen determinations. Some of these key characteristics are discussed, but no data is provided for this specific talc powder. Lastly, to clearly show that these cells were malignant, an in vivo experiment such as subcutaneous injection of these transformed cells into an immune compromised mouse model, would need to be conducted showing tumor growth and ability to invade neighboring tissue. All claims for 'malignant transformation' should be changed to 'cell transformation.'
6) Page 3, Lines 19-20. The statement 'Previous in vitro studies have demonstrated a biologic effect when cells in culture are exposed to talc' is very generalized and vague. Please give specifics on what was previously reported in the literature.
7) The authors tested a lot of Johnson and Johnson talc powder that was not from a previously identified lot that contained asbestos fibers. Since the authors refer to asbestos fibers in talc powder possibly causing ovarian cancer and asbestos fibers are a known carcinogen, it is surprising that no particle characterization effort was performed. No polarized light or electron microscopy of talc particles was performed to investigate potential existence of fibers in the talc powder samples. In addition, what type of TiO2 was tested? Where was it acquired from? Most particle-based toxicity studies are now recommended to perform minimal particle characterization (size, shape, density, elemental analysis, surface area, surface reactivity, etc) as part of a published study since these factors contribute to toxicological response. Lastly, since this is a particle exposure conducted on a submerged in vitro culture, an estimate of deposited or internal dose should have been performed. The effective density of particles determined by particle characteristics, medium type, protein corona, etc can influence particle kinetics and cause differences in the deposited dose that the cells come into contact with.
8) The authors supply no justification for their chosen dose range. Is this a range that is expected in ovarian tissue with topical external application of talc powder? At what dose does talc become acutely toxic to ovarian cells? Please provide

this justification.
9) The method for particle exposure to ovarian cells is not adequate. What was the seeding density in each well and what size wells were used? What size were the talc powder particles and TiO2 particles in cell culture medium during exposure? How were exposed cells handled and transferred to the colony formation assay? Were the cells washed to remove any residual particle prior to agar plating? Figure 2 shows 'control' cells and 'negative control' cells? It was never clearly described what the difference between these treatment groups were. Please provide. Page 5 states that the negative control was a blank. If so, how can you have a positive percent transformed cell response for this treatment?
10) Page 5. The authors did not clearly state what the final concentration of exposed ovarian cells were in each well for the colony formation assay. This is critical since agar-based colony formation assays are susceptible to starting cell density. The authors state 'test cells were all made as 30 thousand cells/well' in the paragraph describing the standard curve method and again in Figure 2. Figure 3 left hand panels show a high density of cells in agar. Was this the colony transformation assay seeding density in each 96 well plate? If 30,000, this is a VERY large number since many previous publications using similar soft agar and agarose assays typically use ~1,000 cells per cm2. The methods described here suggest a 90,000 cells per cm2 seeding density. A low seeding density will test an individual cell's ability to survive non-attachment to substrate or other cells and grow into a suspended colony. I would guess that 30,000 cells were highly confluent in a 96-well plate and able to rely on each other for survival. These authors previously reported that talc-exposed ovarian cells showed increased oxidative stress, proliferation, and some resistance to apoptosis. Near confluent, stressed epithelial cells can clump together in culture, but this is more of a stress response and not an indication of cell transformation associated with tumorigenesis. Other endpoints showing a transformed phenotype is needed. Lastly, transformed formazan dye is the endpoint and not a colony count. Hence high seeding density resulting in surviving cells not in a true colony is possible, and was not clear from the presented data. Since this the only data set supplied in this study, the transformation conclusion based on the results from unclear methodology is highly worrisome.
11) Based on the experimental design and presented data, a student t-test is an inappropriate test for this data set. A two or three-way analysis of variance (ANOVA) with dose, particle, and cell type would be a more appropriate statistical analysis. Did the authors test the assumptions to run a parametric statistical test? In addition, it is not clear what 'percent of transformed cells' refers to in Figure 2? What treatment was used as a control to determine xero%? It was not clearly described how percent of transformed cells was calculated? Please address.
12) Page 3. The authors state 'several animal studies have reported that talcum powder causes inflammation'. The cited studies are not animal studies; they are human patient studies. Furthermore, the Wong et al. is a human patient study that concluded there was no link between talc use and ovarian cancer. In addition, the next sentence refers to in vitro studies and refers to large scale reviews. If animal and in vitro studies with specific effects need to be cited, please cite this primary literature and describe with enough detail to support the background argument.

Minor Comments
1) Minor grammar errors were found throughout the manuscript. Please carefully review the entire manuscript.
2) Ug/ml is used instead of µg/ml. Please address all occurrences.
3) Reference #34 is the same as #3. Please omit. The first statement of the first full paragraph on Page 9 will need another source.

Reviewer #2: In this submission, Ghassan et al. aim to convince the reader that exposure of ovarian cells (both primary and non-primary) to talc powder leads to malignant transformations. However, this paper is written in such a manner, that the science cannot be trusted.

1. Comments on abstract:
a. In the second line, there should be a comma after the word "Here". It should read "Here, we determined…"
b. In the literature , cells are more commonly described with the word "normal" being used first. Consider saying "normal human ovarian cell", "normal human primary ovarian cell" etc.
c. Sixth line down, when mentioning doses, suggest re-wording to say " …were treated with either 100 or 500 ug/ml…."
d. Last paragraph of abstract, second sentence. Suggested re-wording "These findings represent…."
e. I would caution against using the word genital, as the reader would assume that would also include peritoneal fibroblast, but no transformations were observed in this cell line. Or perhaps, specify again that this was specific to the ovarian cells, but not fibroblasts.

2. Comments on Introduction
a. Second sentence needs re-wording. This reads better "…presents with various histopathologies, molecular biologies, and clinical outcomes, and is therefore considered…."
b. Should there be a word before screening methods? Last sentence of the first paragraph in the introduction. As written, it sounds like there is a lack of screening methods. If this is what the authors are trying to say, then it is fine.
c. Consider the word "Eventual" before "development of chemoresistance"
d. "The pathogenesis of EOC remains elusive…."
e. Second paragraph, second sentence that begins with "epithelial ovarian cancer cells…" Are the authors referring to in vitro studies, in vivo studies, human studies? Please be more specific. The same goes for the sentence after that, please describe this in a more specific manner.
f. Third paragraph of introduction, the word "Have" needs to be inserted after "meta-analyses".
g. The first two sentences of the third paragraph of the introduction essentially say the same thing, consider re-writing this.
h. In the animal studies described in the introduction, do the authors mean through dermal exposure? In general, the introduction lacks a lot of important specifics that would improve the quality of this submission.
i. The sentence that starts with "previous in vitro studies" does not mention the cell type or anything important information that the readers needs.
j. The last paragraph of the introduction is actually the most important, but the authors have written it in such a way that leaves more questions than answers. First off, silica and asbestos toxicity should get more attention in the introduction.

Second, why are the authors telling us about asbestos fibers in the lung? This paper is about the genital use of talc powder, so please focus on that, or at least say that it could possibly initiate a similar inflammatory response in ovarian and fibroblast cells. However, the mechanism of cell toxicities in the lung are going to be vastly different that those observed in ovarian and fibroblast cells, so this last paragraph needs to be refined considerably.

3. Materials and Methods
a. This section needs major revisions. Please make sub-categories within this section. For example, the primary human ovarian cells need their own sub-section, with a more detailed explanation of culture methods. Same goes for all cell lines, followed by a separate section for materials used (i.e. talc powder), and each method also needs its own sub-section (Cell Transformation Assay, WST etc..)
b. Primary cells, in particular, require extra care and effort. The authors, for all cell lines, have just glazed over culture methods. What was the temperature and humidity and %CO2 in which these cells were kept? What extra steps were taken with the primary cells?
c. Why was titanium dioxide chosen as a control? Reader needs to know this
d. The lack of detail in this section makes this paper extremely difficult to follow. Please see all questions below
General
i. How were the doses of 100 ug/ml and 500 ug/ml chosen?
ii. Where is the explanation that these doses are even relevant for the exposure model?
iii. Where was the titanium dioxide obtained from?
iv. Were the "suspensions" of talcum baby powder or titanium dioxide prepared freshly every time, or were they stored (either at room temp or in fridge)?
v. What were the sonicator settings? What brand sonicator?
vi. A much more detailed explanation of how cells were maintained and cultured/sub-cultured is needed.

Cell-transformation Assay
vii. The authors fail to tell the reader what density of cells were used for these experiments. 24 wells? 96 wells? All relevant information is missing.
viii. Was the medium changed or cells transferred to new plates prior to exposures?
ix. "This assay was chosen because it is more stable, faster…"Please remove this poorly written sentence. Considered re-wording "Compared to the (Traditional?) soft agar assay, this cell transformation assay was chosen because…" Alternately, there is no need to mention a comparison to the soft-agar assay because up until this point, it has not been on the reader's radar.
x. How were the results of the colorimetric cell transformation assay quantified? The authors make no mention of plate reader.
xi. Did the authors leave the talcum powder suspensions or titanium dioxide suspensions in the cell culture wells for 72 hours straight? Or, was the medium replaced and refreshed? This needs to be made very clear
xii. For controls, was cell viability measured over the course of 72 hour exposures to ensure that just sitting for 3 days with/without medium change (hard to tell, no information is given to reader) did not have an effect on viability?

WST Assay
a. Authors says a cell-dose curve was established in manufacturer's protocol. What manufacturer are they referring to?
b. Why is there so much detail in the WST assay, but not elsewhere?
c. Where was WST obtained from?
d. Was the WST assay done only after the 72 hour exposure? I would think that leaving the cells for 72 hours, the authors would check the viability of control cells every 24 hours to ensure that all decreases in viability were from exposures, and not just sitting (authors do not tell us cell culture conditions) for 72 hours.
e. Authors do not tell readers what WST assay is for
f. This entire description needs to be re-done
g. Did the authors look at supernatants? How does that work, is the powder fully dissolved at this point or is it still a suspension? If powder is present in the supernatants tested, that will have an effect on colorimetric assays
h. Were supernatants read right away? Stored for future use?
i. WST-1 is normally used for viability, it is unclear how this played a role in identifying malignancies?

Agarose and WST
a. I have no idea what I am reading here. First the authors talk about an agarose assay, then WST, and now they are combined? This is very poorly explained.

The authors mention malignant transformations observed in cells, but where was the methodology for this?
I did not see any carcinogenic assays
I did not see any methodology for detecting malignant transformations

If the premise of this paper is that talc paper causes cancer, it would make sense that the authors of this paper not only use talc powder, but perhaps try to get the individual components of the talc powder, so they can correctly identify what it is in the talc powder that is cancerous.

RESULTS
If the authors do not tell the reader which one of their methods was used to detect cell malignancies, then how is the reader supposed to just understand this results section?
How exactly were transformed cells quantified? Using the WST-1 assay? Using agarose? WST is traditionally used for

How exactly were transformed cells quantified? Using the WST-1 assay? Using agarose? WST is traditionally used for viability.

How were malignant colonies counted?

How do the authors have images of cells in their data, and make no mention of this in their methodology? Colorimetric assays were mentioned, and reading of absorbance, there was no mention of microscopy work/histology.

The problems with this submission are too numerous to count, and the science , methodology, and data cannot be trusted.

Discussion section cannot be trusted based on all of the numerous issues listed above.

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Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells

--Manuscript Draft--

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Corresponding Author:	Ghassan M Saed, Ph.D. Wayne State University School of Medicine Detroit, Michigan UNITED STATES
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Abstract:	Several epidemiological and molecular studies have linked perineal use of talcum powder to increased risk of ovarian cancer. Here we determined that exposure to talcum powder induces malignant transformation in human normal ovarian cells. Human primary normal ovarian epithelial cells (Cell Biologics), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories), and human primary peritoneal fibroblasts were treated with 100 and 500 ug/ml of talcum powder or titanium dioxide (TiO2) as a control for 72 hours before assessment with a cell transformation assay. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in a dose dependent manner in both ovarian cell lines. No colonies formed in the untreated ovarian cells or control ovarian cells at either dose. Interestingly, there were no colonies formed in talc treated normal peritoneal fibroblasts. Treatment with talc increased number of transformed ovarian cells by 11% and 20% in the 100 and 500 ug/ml doses, respectively ($p<0.05$). Likewise, but to a greater extent, treatment with talcum powder increased number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively ($p<0.05$). There were no detectible transformed cells when treated with TiO2. Exposure to talcum powder induces malignant transformation in normal ovarian epithelial cells but not in normal peritoneal fibroblasts. This finding represents a direct causation mechanism of talcum powder exposure specific to normal ovarian cells and further supports previous studies of the association of genital use of talcum powder and increased risk of ovarian cancer.
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Additional Information:	
Question	**Response**
Financial Disclosure Enter a financial disclosure statement that describes the sources of funding for the	A portion of Dr. Saed's time conducting this research was paid for by the lawyers representing plaintiffs in the talcum powder litigation. Dr. Saed received no financial support for the authorship or publication of this article

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Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells

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Key words: talc, talcum powder, titanium dioxide, epithelial ovarian cancer, transformation, primary cells, cell proliferation, colonies

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Abstract

Several epidemiological and molecular studies have linked perineal use of talcum powder to increased risk of ovarian cancer. Here we determined that exposure to talcum powder induces malignant transformation in human normal ovarian cells.

Human primary normal ovarian epithelial cells (Cell Biologics), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories), and human primary peritoneal fibroblasts were treated with 100 and 500 ug/ml of talcum powder or titanium dioxide (TiO_2) as a control for 72 hours before assessment with a cell transformation assay.

Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in a dose dependent manner in both ovarian cell lines. No colonies formed in the untreated ovarian cells or control ovarian cells at either dose. Interestingly, there were no colonies formed in talc treated normal peritoneal fibroblasts. Treatment with talc increased number of transformed ovarian cells by 11% and 20% in the 100 and 500 ug/ml doses, respectively ($p<0.05$). Likewise, but to a greater extent, treatment with talcum powder increased number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively ($p<0.05$). There were no detectible transformed cells when treated with TiO_2.

Exposure to talcum powder induces malignant transformation in normal ovarian epithelial cells but not in normal peritoneal fibroblasts. This finding represents a direct causation mechanism of talcum powder exposure specific to normal ovarian cells and further supports previous studies of the association of genital use of talcum powder and increased risk of ovarian cancer.

Introduction

Ovarian cancer is a gynecologic malignancy that ranks fifth in cancer deaths among women in United States (*1*). Epithelial ovarian cancer (EOC) presents with various histopathology, molecular biology, and clinical outcome is thus considered a heterogeneous disease (*1, 2*). The prognosis of EOC remains poor, with a 5-year survival rate of 50% in advanced stage (*2*). This is largely due to the lack of early warning symptoms, screening methods, and the development of chemoresistance (*1, 2*).

The pathogenesis of EOC is still being elucidated but has been strongly associated with oxidative stress and inflammation (*3*) (*4*). Epithelial ovarian cancer cells manifest a persistent pro-oxidant state that has been demonstrated to be further enhanced in chemoresistant EOC cells (*5*),(*3*). Attenuation of the pro-oxidant state with antioxidants/scavengers has been shown to selectively induce apoptosis in EOC cells indicating a potential therapeutic value (*5*),(*3*).

The association between genital use of talcum powder and risk of EOC have been previously described (*3, 6, 7*). Several Meta-analyses demonstrated a statistically significant increased risk of ovarian cancer with the genital use of talcum powder (*8-10*). In addition, several animal studies have reported that talcum powder causes inflammation and oxidative stress in animals (*11*) (*12-14*). Previous *in vitro* studies have demonstrated a biologic effect when cells in culture are exposed to talc (*15-18*). In support of these previous findings, we have recently delineated the molecular basis of the association of talcum powder use with increased risk of ovarian cancer (*19*).

On October 18, 2019, Johnson & Johnson recalled Johnson's Baby Powder lot #22318RB following FDA's finding that the product contained chrysotile asbestos. Talc

and asbestos are both silicate minerals; the carcinogenic effects of asbestos have been extensively studied and documented in the medical literature (*3, 6*). Asbestos fibers in the lung initiate an inflammatory and scarring process, and it has been proposed that ground talc, as a foreign body, might initiate a similar inflammatory response (*3*). Here we sought to determine whether exposure to talcum powder will induce malignant transformation in human primary normal ovarian epithelial cells and human primary normal peritoneal fibroblasts.

Material and Methods

Human primary normal ovarian epithelial cells (Cell Biologics, Chicago, IL), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories, Inc, Carlsbad, California), and human primary normal peritoneal fibroblasts were isolated and cultured as we have previously described (*20, 21*). The fibroblast cell line has been extensively characterized in previous studies and has been shown to be pure and solely fibroblast cells (*20, 21*). Human normal ovarian epithelial cells and HOSEpiC cells were grown in complete human epithelial cell medium and conditions following their manufacturer's protocols. Human normal primary peritoneal fibroblasts were grown in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, CA). All media was supplemented with 10 % fetal bovine serum (FBS, Innovative Research, Novi, MI) and penicillin/streptomycin (Fisher Scientific, Waltham, MA), per their manufacturer specifications.

Talcum baby powder (Johnson & Johnson, #30027477, Lot#13717RA) or Titanium dioxide (TiO_2) were suspended in PBS (Stock solution of 50 mg/ml) and sonicated 3 times for 1 minute each. Human primary normal ovarian epithelial cells and human primary

peritoneal fibroblasts were treated in duplicate with 100 and 500 u g/ml of talcum powder or TiO_2 as a control for 72 hours before assessment with cell transformation assay (colorimetric), according to the manufacturer protocol (Abcam-235698, Cambridge, MA). The experiments were repeated 3 times. This assay was chosen because it is more stable, faster and more sensitive than the Soft-Agar Assay.

A Cell-dose curve was established as described in the manufacturer's protocol. Briefly, we used cells (5.34 x 10^5 cells/ml) were suspended in 1X DMEM/10% FBS medium. Cells were diluted into seven serial dilutions in a 1.5 mL centrifuge tubes. Serial dilution was performed using an 8 channel multi pipette by adding 150 µl of media to each well of a 96 well microplate. A 150 µl aliquot of the 5.34 x10^5 cells/ml (80 x10^3 cells) was added to the wells of the first duplicate row. A 150 µl aliquot from the first duplicate row was removed and added to the next well and mixed. The process was repeated until the seven serial dilutions were obtained. The final well was for negative control (Blank) with media only and no cells. Test cells were all made as 30 x10^3 cells/well. A 35 µl aliquot of 1X DMEM/10% FBS and 15 µl of WST working solution were then added into each well and incubate at 37° C for 4 hours. The absorbance was measured by a microtiter plate reader at 450 nm (Figure 1).

Agarose and WST working solutions were prepared as described in the kit information sheet. The base agarose mix was added into the required wells and kept for 15 minutes at 4°C to solidify the agarose. Stock solution was prepared by using a solution of cells (1 X 10^6 cells/mL in 1X DMEM/10% FBS medium). The agarose-cell mix was added into every well of a 96 well plate previously holding the solidified base agarose layer and placed at 4°C for 10 minutes to solidify the layer. After placing the plate for 10

min at 37ºC, 1X DMEM/10% FBS medium was added to all the wells and incubated at 37°C for 6-8 days. On the last day the upper medium on the top agarose layer was cautiously removed by pipetting. A 1X DMEM/10% FBS and WST working solution was added into each well, incubated for 4 hours at 37°C. The absorbance was measured by a microtiter plate reader at 450 nm.

Statistical Analysis: Data was analyzed with paired t-tests. Data are expressed, as means +/- SD. Significance was determined as $P < 0.05$.

Results

Treatment with talcum powder significantly increased number of transformed normal epithelial ovarian cells by 11% and 20% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, $p<0.05$). Likewise, but to a greater extent, treatment with talcum powder significantly increased number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, $p<0.05$). Talcum powder had no detectable transformation effect on normal peritoneal fibroblasts (Figure 2). Anchorage-independent growth is a hallmark of cancer cells. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in both of the normal epithelial ovarian cell lines in a dose dependent manner (Figure 3). Interestingly, there were no colonies formed in talcum powder treated normal fibroblasts (Figure 3). There were no colonies formed in either untreated ovarian cells or control ovarian cells at either dose. There were no detectible transformed cells when cells were treated with the control, TiO_2.

Discussion

This is the first study to directly show that exposure to talcum powder induces malignant transformation in normal human ovarian epithelial cells. Interestingly, the ability of talcum powder exposure to induce transformation appears to be specific to ovarian cells as it did not induce transformation in human normal peritoneal fibroblasts. (Figure 3). The reason for this specific effect of talcum powder on the ovaries in still under investigation. Talc and asbestos particles have been detected in pelvic lymph nodes and other pelvic organs including the ovaries (*3, 6, 22-24*). Several studies have pointed toward the peristaltic pump feature of the uterus and fallopian tubes, which is known to enhance transport of sperm into the oviduct ipsilateral to the ovary bearing the dominant follicle (*6, 7, 25*).

The first epidemiologic study suggesting an increased risk of ovarian cancer with the genital use of talcum powder was published by Cramer et al. in 1982. These studies have shown the development of lung tumors in female, not male rats exposed to talc (*26*). Additional studies have shown some tumor-like morphological changes and macrophage activation as a result of talc exposure (*26-28*). However, it has been reported that inhalation of talc for a prolonged time by experimental animals did not induce cancer (*28-30*). Collectively, these findings seem to suggest that direct contact of talc with ovarian cells is required to enhance the process of cellular transformation.

The epidemiologic association of talcum powder use and risk of ovarian cancer has been established (*31, 32*). The risks for ovarian cancer from genital talcum powder use vary by histologic subtype, menopausal status at diagnosis, hormone therapy use, weight, and smoking, however, necessary information about the frequency and duration

of usage were lacking (*31-33*). Macrophage activation and inflammatory response to talcum powder were suggested as a link to increased risk of ovarian cancer.

Studies of other sources and batches of talcum powder as well as with other cell types are needed for a more comprehensive evaluation of the effect. Additional animal studies could further support the extent the effect of talcum powder. However, the result of this study clearly supports the molecular and epidemiological data on talc powder use and ovarian cancer risk.

Oxidative stress and inflammation have been implicated in the pathogenesis of ovarian cancer, specifically, by increased expression of several key pro-oxidant enzymes in EOC tissues and cells as compared to normal cells indicating an enhanced redox state (*34*). This redox state is further enhanced in chemoresistant EOC cells as evidenced by a further increase in key pro-oxidant enzymes and a decrease in anti-oxidant levels, suggesting a shift towards a pro-oxidant state (*34*). Antioxidant enzymes, key regulators of cellular redox balance, are differentially expressed in various cancers, including ovarian (*34, 35*).

Our laboratory was the first to confirm the cellular effect of talcum powder and provide a potential molecular mechanism. Talcum powder exposure induced molecular changes in redox enzymes in normal ovarian cells similar to those known for ovarian cancer(*3, 19*). In all talc-treated cells, there was a significant dose-dependent increase in key prooxidants with a concomitant decrease in key antioxidants enzymes. Remarkably, talcum powder exposure induced specific point mutations that are known to alter the activity in some of these key enzymes. The mechanism by which talcum powder alters the cellular redox and inflammatory balance involves the induction of specific mutations

in key oxidant and antioxidant enzymes that correlate with alterations in their activities. The fact that these mutations happen to correspond to known SNPs of these enzymes suggests a genetic predisposition to developing ovarian cancer with genital talcum powder use.

We have previously reported that EOC cells manifest increased cell proliferation and decreased apoptosis (*34*). Recently, we have shown that talcum powder enhances cell proliferation and induces an inhibition in apoptosis in EOC cells, but more importantly in normal cells, suggesting talc is a stimulus to the development of the oncogenic phenotype. Talcum powder exposure also resulted in a significant increase in inflammation as determined by increased tumor marker CA-125. These findings are the first to confirm the cellular effect of talcum powder and provide a molecular mechanism to previous reports linking genital use to increased ovarian cancer risk.

Declaration of Conflicting Interests:

Dr. Saed has served as a paid consultant and expert witness for the plaintiffs in the talcum powder litigation. The remaining authors have no potential conflicts of interest to report.

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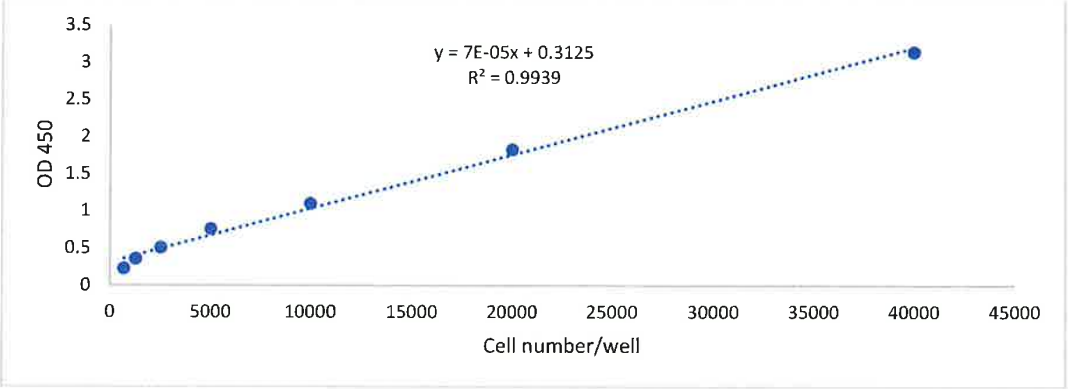

Figure 1. Human primary normal ovarian epithelial cell-dose curve

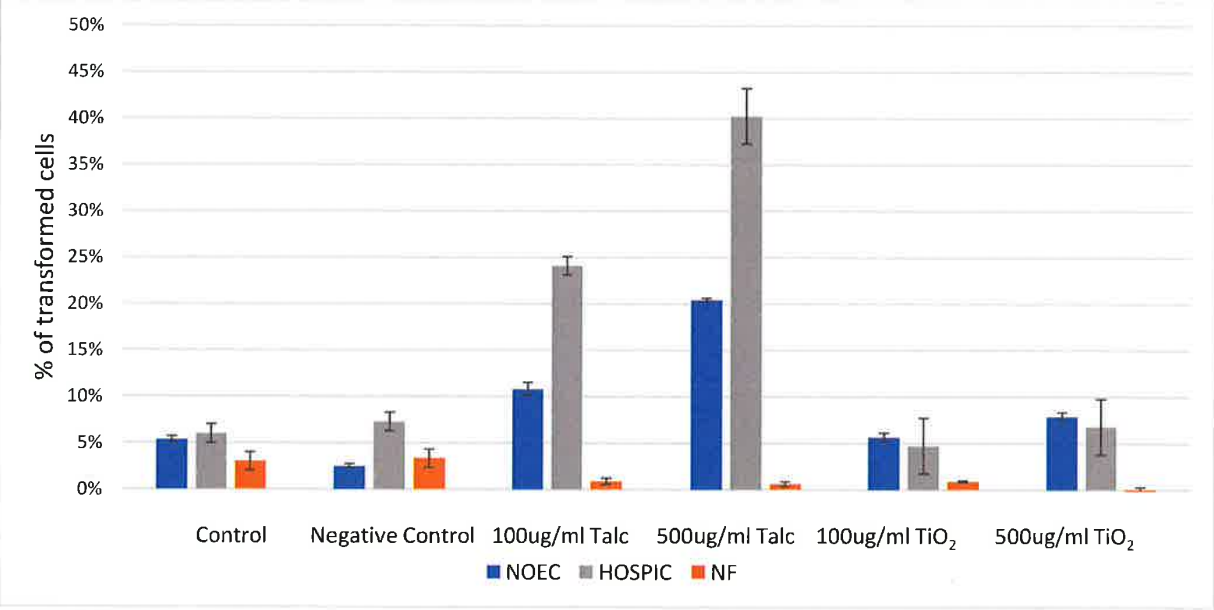

Figure 2. Equal numbers (30K) of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) were seeded for the cell transformation assay as described in methods. After 6 days, the cell number were measured. Standard and samples readings were taken 4 hours after adding WST working solution.

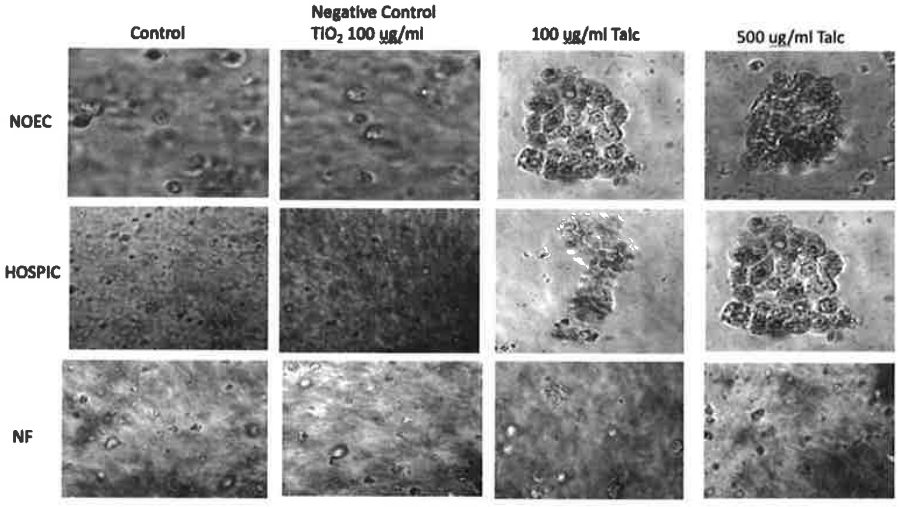

Figure 3: Images of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) treated with 100 and 500 ug/ml of talcum powder, after 6 days of culture.

From: **Reproductive Sciences - Editorial Office** em@editorialmanager.com
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Date: August 27, 2020 at 12:24 AM
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We have received the reports from our advisors on your manuscript RESC-D-20-00635 "Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells".

With regret, I must inform you that, based on the advice received, I have decided that your manuscript cannot be accepted for publication in Reproductive Sciences.

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With kind regards,

Setsuko Chambers
Associate Editor
Reproductive Sciences

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1-The findings still would not establish that it is biologically plausible that talc causes ovarian cancer in living humans .
2- Injection talcum powder into the reproductive systems of laboratory animals to see if the same changes occur is highly recommended.
3-What are the Justification for the Talcum powder amount added to the cell cultures
Are those amounts are in actual situations are present at the commercially used powder and how long the human needs to get these amounts in actual life .
4-Does the talcum powder used is pure or contains traces of asbestos
5-The discussion is lengthy and redundant and the authors keep repeating the same information and self citation without adding any more relevant data or comparisons with several other published data in the same field .

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Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells

--Manuscript Draft--

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Talcum powder induces malignant transformation in human primary normal ovarian epithelial cells

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Abstract

Several epidemiological and molecular studies have linked perineal use of talcum powder to increased risk of ovarian cancer. Here we determined that exposure to talcum powder induces malignant transformation in human normal ovarian cells. Human primary normal ovarian epithelial cells (Cell Biologics), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories), and human primary peritoneal fibroblasts were treated with 100 and 500 u g/ml of talcum powder or titanium dioxide (TiO_2) as a control for 72 hours before assessment with cell transformation assay. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in a dose dependent manner in both ovarian cell lines. There were no colonies formed in untreated ovarian cells or control ovarian cells at either dose. Interestingly, there were no colonies formed in talc treated normal peritoneal fibroblasts. Treatment with talc increased number of transformed ovarian cells by 11% and 20% in the 100 and 500 ug/ml doses, respectively ($p<0.05$). Likewise, but to a greater extent, treatment with talcum powder increased number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively ($p<0.05$). There were no detectible transformed cells when treated with TiO_2. Exposure to talcum powder induces malignant transformation in normal ovarian epithelial cells but not in normal peritoneal fibroblasts. This finding represents a direct causation mechanism of talcum powder exposure specific to normal ovarian cells and further supports previous studies of the association of genital use of talcum powder and increased risk of ovarian cancer.

Keywords: talc, talcum powder, titanium dioxide, epithelial ovarian cancer, transformation, primary cells, cell proliferation, colonies

Introduction

Ovarian cancer is a gynecologic malignancy that ranks fifth in cancer deaths among women in United States [1]. Epithelial ovarian cancer (EOC) presents with various histopathology, molecular biology, and clinical outcome is thus considered a heterogeneous disease [1, 2]. The prognosis of EOC remains poor, with a 5-year survival rate of 50% in advanced stage [2]. This is largely due to the lack of early warning symptoms, screening methods, and the development of chemoresistance [1, 2]. The pathogenesis of EOC is still being elucidated but has been strongly associated with oxidative stress and inflammation.

Epithelial ovarian cancer has been strongly associated with oxidative stress and inflammation [3] [4]. Epithelial ovarian cancer cells manifest a persistent pro-oxidant state that is further enhanced in chemoresistant EOC cells [5],[3]. Attenuation of the pro-oxidant state with antioxidants/scavengers have been shown to selectively induce apoptosis in EOC cells indicating a potential therapeutic value [5],[3].

The association between genital use of talcum powder and risk of EOC have been previously described [3, 6, 7]. Several Meta-analyses demonstrated a statistically significant increased risk of ovarian cancer with the genital use of talcum powder [8-10]. In addition, several animal studies have reported that talcum powder causes inflammation and oxidative stress in animals [11] [12-14]. Previous *in vitro* studies have demonstrated a biologic effect when cells in culture are exposed to talc [15-18]. In support of these previous findings, we have recently delineated the molecular basis of the association of talcum powder use with increased risk of ovarian cancer [19].

On October 18, 2019, Johnson & Johnson recalled Johnson's Baby Powder lot #22318RB following FDA's finding that the product contained chrysotile asbestos. Talc and asbestos are both silicate minerals; the carcinogenic effects of asbestos have been extensively studied and documented in the medical literature [3, 6]. Asbestos fibers in the lung initiate an inflammatory and scarring process, and it has been proposed that ground talc, as a foreign body, might initiate a similar inflammatory response [3]. Here we sought to determine whether exposure to talcum powder will induce malignant transformation in human primary normal ovarian epithelial cells and human primary normal peritoneal fibroblasts.

Material and Methods

Human primary normal ovarian epithelial cells (Cell Biologics, Chicago, IL), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories, Inc, Carlsbad, California), and human primary normal peritoneal fibroblasts were isolated and cultured as we have previously described [20, 21] . The fibroblast cell line has been extensively characterized in previous studies and has been shown to be pure and solely fibroblast cells [20, 21]. Human normal ovarian epithelial cells and HOSEpiC cells were grown in complete human epithelial cell medium and conditions following their manufacturer's protocols. Human normal primary peritoneal fibroblasts were grown in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, CA). All media was supplemented with 10 % fetal bovine serum (FBS, Innovative Research, Novi, MI) and penicillin/streptomycin (Fisher Scientific), per their manufacturer specifications.

Talcum powder or Titanium dioxide (TiO_2) were suspended in PBS (Stock solution of 50 mg/ml) and sonicated 3 times for 1 minute each. Human primary normal ovarian epithelial cells and human primary peritoneal fibroblasts were treated in duplicate with 100 and 500 u g/ml of talcum powder or TiO_2 as a control for 72 hours before assessment with cell transformation assay (colorimetric), according to the manufacturer protocol (abcam 235698). The experiments were repeated 3 times. This assay was chosen because it is more stable, faster and more sensitive than the Soft-Agar Assay.

Cell-dose curve was established as described in manufacturer protocol. Briefly, we used cells (5.34 x 10^5 cells/ml) in 1X DMEM/10% FBS medium and a control containing no cells. Cells were diluted into seven serial dilutions in a 1.5 mL centrifuge tubes. Serial dilution was performed using an 8 channel multi pipette by adding 150 µl of media to each well of a 96 well microplate. One hundred fifty µl aliquot of 5.34 x10^5 cells/ml (80 x10^3 cells) was added to the wells of the first duplicate row. One hundred fifty µl aliquot from the first duplicate row was removed and added to the next well and mixed. The process was repeated until the seven serial dilutions were obtained. The final well was for negative control (Blank) with media only and no cells. Test cells were all made as 30 x10^3 cells/well. A 35 µl aliquot of 1X DMEM/10% FBS and 15 µl of WST working solution were then added into each well and incubate at 37° C for 4 hours. The absorbance was measured by a microtiter plate reader at 450 nm.

Agarose and WST working solutions were prepared as described in the kit information sheet. The base agarose mix was added into the required wells and kept for 15 minutes at 4°C to solidify the agarose. Stock solution was prepared by using a solution of cells (1 X 10^6 cells/mL in 1X DMEM/10% FBS medium). The agarose-cell mix was

added into every well of a 96 well plate previously holding the solidified base agarose layer and placed at 4°C for 10 minutes to solidify the layer. After placing the plate for 10 min at 37ºC, 1X DMEM/10% FBS medium was added to all the wells and incubated at 37°C for 6-8 days. On the last day the upper medium on the top agarose layer was cautiously removed by pipetting. A 1X DMEM/10% FBS and WST working solution was added into each well, incubated for 4 hours at 37°C. The absorbance was measured by a microtiter plate reader at 450 nm.

Statistical Analysis: Data was analyzed with paired t-tests. Data are expressed, as means +/- SD. Significance was determined as $P < 0.05$.

Results

Treatment with talcum powder significantly increased number of transformed normal epithelial ovarian cells by 11% and 20% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 3, $p<0.05$). Likewise, but to a greater extent, treatment with talcum powder significantly increased number of transformed HOSEpiC cells by 24% and 40% in the 100 and 500 ug/ml talcum powder doses, respectively (Figure 2, $p<0.05$). Talcum powder has no detectable transformation effect on normal peritoneal fibroblasts (Figure 2). Anchorage-independent growth is a hallmark of cancer cells. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in both of the normal epithelial ovarian cell lines in a dose dependent manner (Figure 3). There were no colonies formed in untreated ovarian cells or control ovarian cells at either dose. There were no detectible transformed cells when cells were treated with TiO_2. Interestingly, there were no colonies formed in talcum powder treated normal fibroblasts (Figure 3).

Discussion

This is the first study to directly show that exposure to talcum powder induces malignant transformation in normal human ovarian epithelial cells. Interestingly, the ability of talcum powder exposure to induce transformation appears to be specific to ovarian cells as it did not induce transformation in human normal peritoneal fibroblasts. (figure 3). The reason for this specific effect of talcum powder on the ovaries in still under investigation. Talc and asbestos particles have been detected in pelvic lymph nodes and other pelvic organs including ovaries [6, 3, 22-24]. Several studies have pointed toward the peristaltic pump feature of the uterus and fallopian tubes, which is known to enhance transport of sperm into the oviduct ipsilateral to the ovary bearing the dominant follicle [6, 7, 25].

The first epidemiologic study suggesting an increased risk of ovarian cancer with the genital use of talcum powder was published by Cramer et al. in 1982. These studies have shown the development of lung tumors in female, not male rats exposed to talc [26]. Additional studies have shown some tumor-like morphological changes and macrophage activation as a result of talc exposure [27, 26, 28]. However, it has been reported that inhalation of talc for a prolonged time by experimental animals did not induce cancer [28-30]. Collectively, these findings seem to suggest that direct contact of talc with ovarian cells is required to enhance the process of cellular transformation.

The epidemiologic association of talcum powder use and risk of ovarian cancer has been established [31, 32]. The risks for ovarian cancer from genital talcum powder use vary by histologic subtype, menopausal status at diagnosis, hormone therapy use, weight, and smoking however, necessary information about the frequency and duration

of usage were lacking [32, 31, 33]. Macrophage activation and inflammatory response to talcum powder were suggested as a link to increased risk of ovarian cancer.

Studies of other sources and batches of talcum powder as well as with other cell types are needed for a more comprehensive evaluation of the effect. Additional animal studies could further support the extent the effect of talcum powder. However, the result of this study clearly supports the molecular and epidemiological data on talc powder use and ovarian cancer risk.

Oxidative stress and inflammation have been implicated in the pathogenesis of ovarian cancer, specifically, by increased expression of several key pro-oxidant enzymes in EOC tissues and cells as compared to normal cells indicating an enhanced redox state [3]. This redox state is further enhanced in chemoresistant EOC cells as evidenced by a further increase in key pro-oxidant enzymes and a decrease in anti-oxidant levels, suggesting a shift towards a pro-oxidant state [3]. Antioxidant enzymes, key regulators of cellular redox balance, are differentially expressed in various cancers, including ovarian [3, 34].

Our laboratory was the first to confirm the cellular effect of talcum powder and provide a potential molecular mechanism. Talcum powder exposure induced molecular changes in redox enzymes in normal ovarian cells similar to those known for ovarian cancer[3, 19]. In all talc-treated cells, there was a significant dose-dependent increase in key prooxidant with a concomitant decrease in key antioxidants enzymes. Remarkably, talcum powder exposure induced specific point mutations that are known to alter the activity in some of these key enzymes. The mechanism by which talcum powder alters the cellular redox and inflammatory balance involves the induction of specific mutations

in key oxidant and antioxidant enzymes that correlate with alterations in their activities. The fact that these mutations happen to correspond to known SNPs of these enzymes indicate a genetic predisposition to developing ovarian cancer with genital talcum powder use.

We have previously reported that EOC cells manifest increased cell proliferations and decreased apoptosis [3]. Recently, we have shown that talcum powder enhances cell proliferation and induces an inhibition in apoptosis in EOC cells, but more importantly in normal cells, suggesting talc is a stimulus to the development of the oncogenic phenotype. Talcum powder exposure also resulted in a significant increase in inflammation as determined by increased tumor marker CA-125. These findings are the first to confirm the cellular effect of talcum powder and provide a molecular mechanism to previous reports linking genital use to increased ovarian cancer risk.

Declaration of Conflicting Interests:

Dr. Saed has served as a paid consultant and expert witness for the plaintiffs in the talcum powder litigation. The remaining authors have no potential conflicts of interest to report.

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Figure

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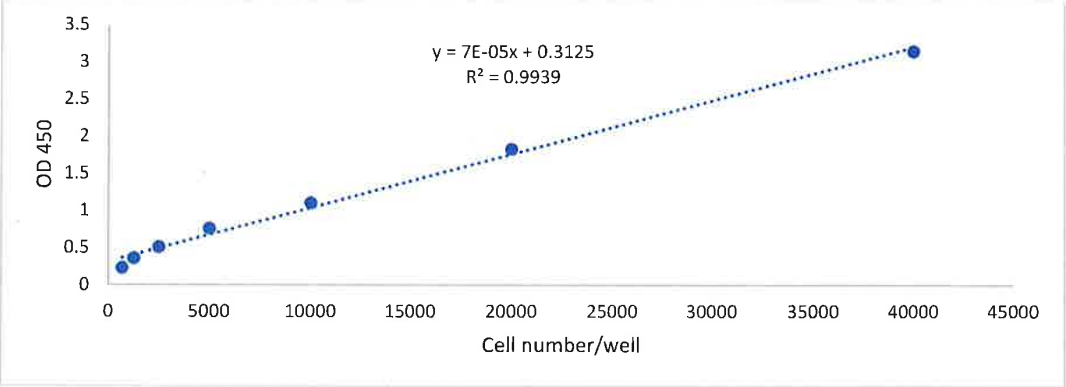

Figure 1. Human primary normal ovarian epithelial cell-dose curve

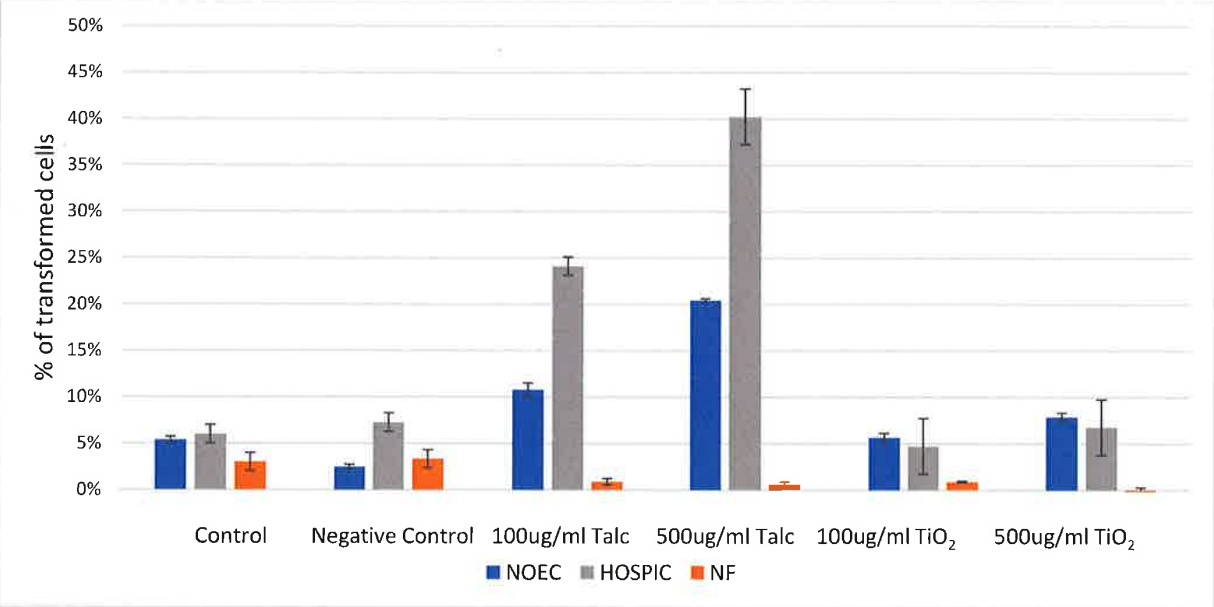

Figure 2. Equal numbers (30K) of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) were seeded for the cell transformation assay as described in methods. After 6 days, the cell number were measured. Standard and samples readings were taken 4 hours after adding WST working solution.

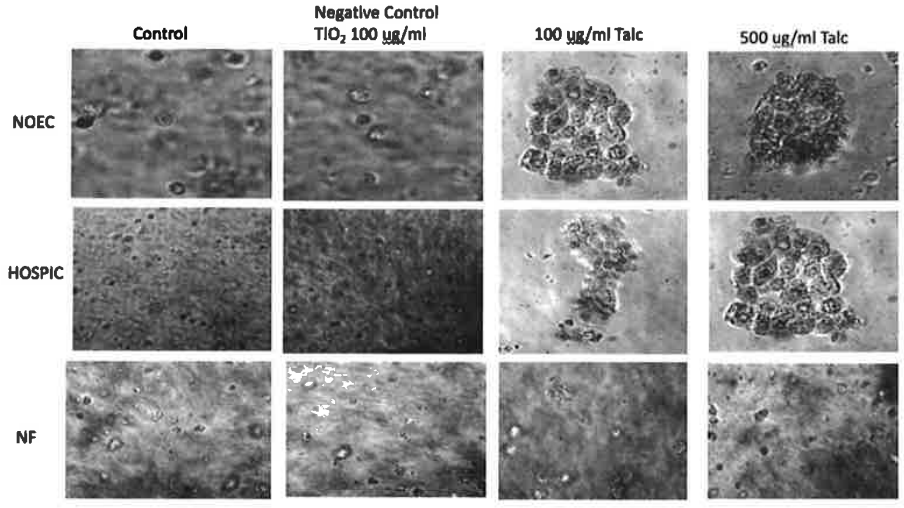

Figure 3: Images of human primary normal ovarian epithelial cells (NOEC), human ovarian epithelial cells (HOSEpiC) and human normal peritoneal fibroblast cells (NF) treated with 100 and 500 ug/ml of talcum powder, after 6 days of culture.

ethical consents

Click here to access/download;ethical consents;This is an observational study.docx

This is an observational study which utilizes commercially available cell lines. No ethical approval is required.

From: **Dale Gibbons** dale.gibbons@sgo.org
Subject: Abstract #15665 for SGO 2020 Annual Meeting
Date: January 31, 2020 at 8:31 AM
To: aharper4@med.wayne.edu, florrie1522@gmail.com, gb1258@wayne.edu, nicolefletcherking@gmail.com, rmorris@med.wayne.edu, gsaed@med.wayne.edu
Cc: Kim, Kenneth kkim@uabmc.edu, Moore, Kathleen N. (OB/GYN - HSC) Kathleen-Moore@ouhsc.edu

DG

Dear Drs. Harper, Fan, Majed, King, Morris, and Saed,

I am writing to you on behalf of SGO's 2020 Annual Meeting Program Committee Co-chairs, Drs. Kathleen Moore and Kenneth Kim. Drs. Moore and Kim would like to know if you would like to update your disclosures for the attached abstract. Please let me know as soon as possible so we can accurately report the disclosures in the printed program book.

You may email any updates directly to me.

Please contact me if you have any questions.

Thank you,

Dale

Dale Gibbons, CAE
DIRECTOR OF PROFESSIONAL EDUCATION | SOCIETY OF GYNECOLOGIC ONCOLOGY | FOUNDATION FOR WOMEN'S CANCER
dale.gibbons@sgo.org | sgo.org | foundationforwomenscancer.org
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297 - Talcum powder induces malignant transformation of human primary normal ovarian epithelial cells but not human primary normal peritoneal fibroblasts

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Sunday, March 29, 2020 : 3:30 PM - 5:00 PM

Monday, March 30, 2020 : 10:15 AM - 11:15 AM

Monday, March 30, 2020 : 3:30 PM - 5:00 PM

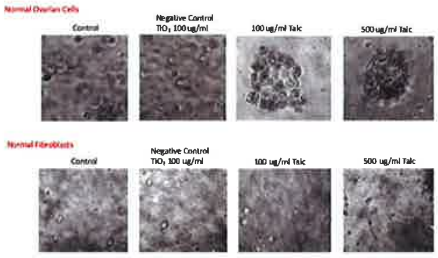
Abstract

Objective: The aim of this study was to demonstrate whether exposure to talcum powder induces malignant transformation in human primary normal ovarian epithelial cells and human primary normal peritoneal fibroblasts. Epidemiological and molecular studies have linked perineal use of talcum powder to increased risk of ovarian cancer. Exposure to talcum powder was shown to induce specific point mutations in key redox enzymes that altered their activities in both normal and epithelial ovarian cancer cells.

Method: Talcum baby powder (Johnson & Johnson, N.J. #30007477, Lot#13717RA) or titanium dioxide (TiO_2, Fischer Scientific) were suspended in PBS (stock solution of 50 mg/ml) and sonicated 3 times for 1 minute each. Human primary normal ovarian epithelial cells (Cell Biologics)and human primary peritoneal fibroblasts were treated in triplicate with 100 and 500 ug/ml of talcum powder or TiO_2 as a control for 72 hours before assessment with cell transformation assay (Abcam), according to the manufacturer protocol. This assay was chosen because it is faster, stable, and more sensitive than the traditional Soft-Agar Assay.

Results: Anchorage-independent growth is a hallmark of cancer cells. Treatment with talcum powder resulted in formation of colonies, indicating cell malignant transformation in a dose-dependent manner. There were no colonies formed in untreated ovarian cells or control ovarian cells at either dose. Interestingly, there were no colonies formed in normal fibroblasts treated with talcum powder (Fig. 1). Treatment with talcum powder significantly increased number of transformed ovarian cells by 11% and 20% in the 100 and 500 ug/ml doses, respectively ($P < 0.05$). There were no detectible transformed cells when treated with TiO_2. Data were analyzed with paired t tests.

Conclusion: Exposure to talcum powder induces malignant transformation in normal ovarian epithelial cells but not in normal peritoneal fibroblasts. This finding represents a direct causation mechanism of talcum powder exposure specific to normal ovarian cells and further supports previous studies of the association of genital use of talcum powder and increased risk of ovarian cancer.



Authors

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Medpace, Inc.

Robert T. Morris
Barbara Ann Karmanos Cancer Institute/Wayne State University School of Medicine

Ghassan M Saed
Barbara Ann Karmanos Cancer Institute

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[1]Osaka University, Osaka, Japan, [2]University of Southern California, Los Angeles, CA, USA, [3]Tohoku University School of Medicine, Miyagi Prefecture, Japan, [4]Toho University School of Medicine, Kanagawa, Japan, [5]National Hospital Organization Kyushu Cancer Center, Fukuoka Prefecture, Japan, [6]Osaka International Cancer Institute, Osaka, Japan, [7]Keio University, School of Medicine, Tokyo, Japan, [8]Iwate Medical University School of Medicine, Morioka, Japan

Histopathological characterization of the tubal fimbria reveals a subgroup of *BRCA1* mutation carriers with tumor-promoting gene-set enrichment

Y. Raz[1,2], B. Tabori[3], B. Taylor-Harding[3], A.E. Watts[3], B.Y. Karlan[3] and S. Orsulic[3,4]
[1]Los Maternity Hospital - Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel, [2]Womens Cancer Program Cedars-Sinai Medical Center, Los Angeles, CA, USA, [3]National Cancer Institute, Bethesda, MD, USA, [4]Cedars-Sinai Medical Center, Los Angeles, CA, USA, [5]David Geffen School of Medicine at UCLA, Los Angeles, CA, USA, [6]University of California, Los Angeles, Los Angeles, CA, USA

From: sgo@confex.com
Subject: SGO 2020 Annual Meeting on Women's Cancer: Submission Initiated (id#15665)
Date: September 16, 2019 at 1:17 PM
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Subject: SGO Abstract
Date: September 9, 2019 at 11:13 AM
To: Amy Harper aharper4@med.wayne.edu, Nicole King nfletche@med.wayne.edu, Robert Morris rmorris@med.wayne.edu

I am submitting this abstract to SGO. Please edit and let me know if you have any changes!

> Dr. Ghassan M. Saed
> Associate Professor of Gynecologic Oncology
> Director of Ovarian Cancer Biology Research
> Departments of Obstetrics and Gynecology and Oncology
> Member of Tumor Biology and Microenvironment Program
> Karmanos Cancer Institute
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From: **Robert Morris** rmorris@med.wayne.edu
Subject: Re: Manuscript
Date: July 20, 2020 at 12:38 PM
To: Ghassan Saed gsaed@med.wayne.edu, Amy Harper aharper4@med.wayne.edu, Xin Wang xin.wang7@wayne.edu, N.King@medpace.com N.King@Medpace.com

The manuscript looks great. A couple questions. Why was TiO2 selected as a control? Does this need to be explained in the manuscript? Are the concentrations used physiologically possible (especially in the ovary)?

Bob

From: Ghassan Saed <gsaed@med.wayne.edu>
Sent: Thursday, July 9, 2020 2:55 PM
To: Amy Harper <aharper4@med.wayne.edu>; Robert Morris <rmorris@med.wayne.edu>; Xin Wang <xin.wang7@wayne.edu>; N.King@medpace.com <N.King@Medpace.com>
Subject: Manuscript

Dear All,
The manuscript is ready for submission. Please let me know should you have any corrections or changes. Please use track changes.

Best regards
Ghassan

> Dr. Ghassan M. Saed
> Associate Professor of Gynecologic Oncology
> Director of Ovarian Cancer Biology Research
> Departments of Obstetrics and Gynecology and Oncology
> Member of Tumor Biology and Microenvironment Program
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OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Amy Harper

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Cedarville University, Cedarville, OH	B.S.	05/2009	Biology
University of Toledo College of Medicine and Life Sciences, Toledo, OH	M.D.	06/2014	Medicine
MD Anderson Cancer Center, Felix Rutledge Fellowship, Houston, TX		11/2017	Gynecologic Oncology
The University of Maryland School of Medicine, Obstetrics and Gynecology Residency, Baltimore, MD	Residency	06/2018	Obstetrics and Gynecology
Wayne State University/Karmanos Cancer Institute, Gynecologic Oncology Fellowship, Detroit, MI			Gynecologic Oncology

A. **Personal Statement**

I am currently a fellow in the Gynecologic Oncology fellowship program at Wayne State University/Karmanos Cancer Institute in Detroit, Michigan, where I am completing my training in the clinical care of women with gynecologic malignancies and in the research of this challenging field of medicine. I have participated in several basic science and translational research projects throughout my undergraduate and graduate education, which has prepared me well for taking on more complex research endeavors in fellowship. Having completed a rigorous residency at the University of Maryland in obstetrics and gynecology and the Felix Rutledge Fellowship in gynecologic oncology at the MD Anderson Cancer Center, I am able to relate the data gathered at the laboratory bench to the patients we see in the clinic and on the wards.

As a research fellow, I am directly participating in the development of research strategies, acquisition and interpretation of data, and manuscript writing under the mentorship of the Principal Investigator, Dr. Ghassan Saed. With his guidance and expertise, we have been able to publish in a peer-reviewed journal and present several posters at the Society for Gynecologic Oncology Annual Meeting and the Society for Reproductive Investigation Annual Meeting. I am thrilled to continue to collaborate with Dr. Saed for this exciting and promising proposed investigation.

B. Positions and Honors

2008-2010 Research Assistant, Dept. of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH

2014-2018 Obstetrics and Gynecology Resident, University of Maryland School of Medicine, Baltimore, MD

2018 Administrative Chief Ob/Gyn Resident, University of Maryland School of Medicine, Baltimore, MD

2018-present Gynecologic Oncology Fellow, Wayne State University School of Medicine/Karmanos Cancer Institute, Detroit, MI

Honors:

2013 Gold Humanism Honor Society
2014 Alpha Omega Alpha Honor Medical Society
2017 Felix Rutledge Fellow, MD Anderson Cancer Center, Houston, TX
2018 Resident Research Award, Dept. of Obstetrics and Gynecology, University of Maryland School of Medicine, Baltimore, MD

C. Contributions to Science

1) Effects of alcohol consumption on liver metabolism: Identified the occurrence of a specific damage of hepatic G6Pase following acute alcohol administration. Highlighted the consequence of such damage for the ability of hepatic endoplasmic reticulum to sequester and mobilize its Ca^{2+} store. Identified that acute administration of low doses of EtOH is sufficient to dysregulate Ca^{2+} uptake and cycling within the hepatocyte by impairing the hydrolytic activity of G6Pase and the production of inorganic phosphate within the endoplasmic reticulum. This dysregulation occurs to a greater extent following the acute administration of a high dose of EtOH, as the alcohol not only inhibits the G6Pase activity to a larger extent but also impairs the operation of receptors and pumps within the cell membrane, further compromising Ca^{2+} homeostasis within the hepatocyte. This dysregulation can have severe and far reaching implication for cell bioenergetics (i.e. mitochondrial uncoupling by Ca^{2+}) and gene activation.
Jacobs-Harper, A., Crumbly, A., Romani, A. Acute Effect of Ethanol on Hepatic Reticular G6Pase and Ca2+ Pool. Alcoholism: Clinical and Experimental Research. 37(s1): E40 – E51, January 2013.

2) Implications of EP4 receptor and class III-β tubulin expression on prognosis and treatment of uterine smooth muscle tumors: Class III-b tubulin is known to convey a resistant to taxanes in the treatment of several different cancers. As a mainstay of therapy for gynecologic malignancies, and specifically uterine smooth muscle tumors, this poses a significant hurdle for successful treatment. Receptor EP4 has been identified as an alternative target in a number of different cancers. I investigated the degree of expression of class III-b tubulin and EP4 receptor within human benign and malignant uterine smooth muscle tumors in a large group of human subjects and determined the effect on progression free survival and overall survival. Using commercially available human cell lines, I developed a model for uterine leiomyosarcoma and determined the cytotoxic effect of a proprietary, small molecule drug on this aggressive and devastating malignancy.
Harper, A., Reader, J., Legesse, T., Rao, G., Roque, D., Staats, P. EP4 receptor and class III-β tubulin expression in uterine smooth muscle tumors: Implications for prognosis and treatment. International Gynecologic CancerSociety, 16th Biennial Meeting, Lisbon, Portugal, October 29-31, 2016.

3) Treatment modalities of uterine sarcoma: Uterine sarcomas are characterized by poor response to systemic chemotherapy and high recurrence rates. I investigated whether the use of cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (HIPEC) confers survival benefit in comparison with conventional treatment modalities in patients with recurrent US.
Díaz-Montes TP, El-Sharkawy F, Lynam S, **Harper A**, Sittig M, MacDonald R, Gushchin V, Sardi A. Efficacy of Hyperthermic Intraperitoneal Chemotherapy and Cytoreductive Surgery in the Treatment of Recurrent Uterine Sarcoma. Int J Gynecol Cancer. 2018;28:1130–37. doi: 10.1097/IGC.0000000000001289.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Nicole M. King *(nee Fletcher)*

eRA COMMONS USER NAME (credential, e.g., agency login): aj5932

POSITION TITLE: Postdoctoral Fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Wayne State University, Detroit, MI	B.S.	08/2006	Biological Sciences
Wayne State University School of Medicine, Detroit, MI	Ph.D.	08/2013	Physiology, Concentration in Reproductive Science
Wayne State University School of Medicine, Detroit, MI	Postdoctoral Training	08/2013-Present	Cancer and infertility research

A. Personal Statement

My research has focused on investigating the role of oxidative stress in the pathophysiology of gynecologic fibrotic disorders. Specifically, I have been involved in studying the effects of oxidative stress on the pathogenesis of ovarian cancer as well as in benign overgrowths, such as postoperative adhesions, fibroids, and endometriosis. Our laboratory has identified an important role for myeloperoxidase (MPO) and inducible nitric oxide synthase, key enzymes regulating oxidative stress, in the pathophysiology of ovarian cancer. This work has provided the foundation for this proposal. Specifically, we are the first to report MPO expression in epithelial ovarian cancer cells and tissues, but not in normal epithelial ovarian tissues. Much of my doctoral and postdoctoral work has focused on the role of both oxidants and antioxidants in ovarian cancer as compared to normal ovarian epithelial cells and chemoresistant ovarian cancer cells, and serves as preliminary data and support for this grant proposal. I have experience in performing all assays pertaining to this proposal as evident by the below highlighted studies (1-4). I will execute all experiments as well as writing abstracts, manuscripts, and grants pertaining to this work.

1. Saed GM, Ali-Fehmi R, Jiang ZL, **Fletcher NM**, Diamond MP, Abu-Soud HM, et al. Myeloperoxidase serves as a redox switch that regulates apoptosis in epithelial ovarian cancer. Gynecologic oncology. 2010;116:276-81. PMID: 19962178; PMCID: PMC2834266.

2. Belotte J, **Fletcher NM**, Awonuga AO, Alexis M, Abu-Soud HM, Saed MG, et al. The role of oxidative stress in the development of cisplatin resistance in epithelial ovarian cancer. Reproductive sciences. 2014;21:503-8. PMID: 24077440.

3. Saed GM, Diamond MP, **Fletcher NM**. Updates of the role of oxidative stress in the pathogenesis of ovarian cancer. Gynecol Oncol. 2017 June;145(3):595-602. Epub 2017 Feb 23. Review. PMID: 28237618.

4. Saed GM, **Fletcher NM**, Diamond MP, Morris RT, Gomez-Lopez N, Memaj I. Novel expression of CD11b in epithelial ovarian cancer: potential therapeutic target. *Gynecologic Oncology.* December 2017. *In Press.*

B. Positions and Honors

2002	Dean's list, Wayne State University, Detroit, MI
2002	Blue Cross Blue Shield Superior Academic Performance Scholarship - Winter
2003	Blue Cross Blue Shield Superior Academic Performance Scholarship - Fall
2007-2008	Research Assistant, Wayne State University School of Medicine, Detroit, MI
2008-2013	Interdisciplinary Biological Science Fellowship, Wayne State Univ., Detroit, MI
2008-2013	Graduate Research Assistant, Wayne State University School of Medicine, Detroit, MI
2012-present	Director of Laboratory Operations, DS Biotech, LLC, Detroit, MI
2013-present	Postdoctoral Fellow, Wayne State University School of Medicine, Detroit, MI

C. Below are my significant contributions to science.

1) *The role of oxidative stress in the pathogenesis of ovarian cancer: the discovery of myeloperoxidase expression in ovarian cancer.* We have characterized epithelial ovarian cancer (EOC) cells and tissues to manifest a unique oxidative stress profile, which is further altered in chemoresistance (5). While MPO was previously recognized to be present only in hemopoietic cells, we were the first to report MPO expression in solid ovarian malignancies and EOC cells (6). We have also demonstrated a functional cross talk between MPO and inducible nitric oxide synthase (iNOS), a key oxidant enzyme, where MPO utilizes nitric oxide (NO) as a substrate and converts it to nitrosonium cation, which nitrosylates the caspase-3 active site, leading to inhibition of EOC cells apoptosis, a hallmark of cancer cells (6). Furthermore, reduction of enhanced oxidative stress-induced apoptosis of EOC cells (7). Additionally, we have defined a role for MPO *in vitro* under oxidative stress and *in vivo* in EOC cells to be associated with advanced stage as compared with early stage ovarian cancer (8). More importantly, we demonstrated a significant increase in the levels of MPO and free iron in serum and tissues obtained from early stage ovarian cancer as compared to late stages, as well as compared to benign gynecologic conditions or healthy controls. Collectively, these findings strongly support a role for serum MPO and free iron in the pathophysiology of ovarian cancer, which thereby qualifies it as biomarkers for early detection of ovarian cancer (8). Significantly, the innovative clinical observation is uniquely paired with a molecular biologic process, which provides a plausible mechanism for defining a specific role for these key oxidants enzymes in the pathophysiology of ovarian cancer.

5. Belotte J, **Fletcher NM**, Awonuga AO, Alexis M, Abu-Soud HM, Saed MG, et al. The Role of Oxidative Stress in the Development of Cisplatin Resistance in Epithelial Ovarian Cancer. Reproductive sciences. 2013. PMID: 24077440.

6. Saed GM, Ali-Fehmi R, Jiang ZL, **Fletcher NM**, Diamond MP, Abu-Soud HM, et al. Myeloperoxidase serves as a redox switch that regulates apoptosis in epithelial ovarian cancer. Gynecologic oncology. 2010;116:276-81. PMID: 19962178; PMCID: PMC2834266.

7. Saed GM, **Fletcher NM**, Jiang ZL, Abu-Soud HM, Diamond MP. Dichloroacetate induces apoptosis of epithelial ovarian cancer cells through a mechanism involving modulation of oxidative stress. Reproductive sciences. 2011;18:1253-61. PMID: 21701041.

8. **Fletcher NM**, Jiang Z, Ali-Fehmi R, Levin NK, Belotte J, Tainsky MA, et al. Myeloperoxidase and free iron levels: potential biomarkers for early detection and prognosis of ovarian cancer. Cancer Biomark. 2011;10:267-75. PMID: 22820082.

2) *A Single Nucleotide Polymorphism in Catalase Is Strongly Associated with Ovarian Cancer Survival*. We have clearly established that oxidative stress plays a major role in the pathogenesis of ovarian cancer, however the exact mechanisms remain to be clarified. There is an emerging consensus that most of the genetic component of ovarian cancer risk is due to genetic polymorphisms that confer low to moderate risk (9). Single nucleotide polymorphisms (SNPs) occur because of point mutations that are selectively maintained in

populations that are distributed throughout the human genome at an estimated overall frequency of at least one in every 1000 base pairs (9). Non-synonymous SNPs substitute encoded amino acids in proteins, and are more likely to alter the structure, function, and interaction of the protein (9). Recent evidence demonstrates an association between enzymatic activity altering single nucleotide polymorphisms (SNP) with human cancer susceptibility (10). Chemoresistant ovarian cancer cells manifested specific point mutations, which are associated with altered enzymatic activity, in key redox enzymes that are not detected in sensitive counterparts (9). Supplementation of an antioxidant was able to successfully sensitize ovarian cancer cells to chemotherapeutics. Thus, we have now demonstrated that chemotherapy induces specific point mutations in key redox enzymes, which contributes to the acquisition of chemoresistance in ovarian cancer cells, highlighting a potential novel mechanism (9). Identification of targets for chemoresistance with either biomarker and/or screening potential will have a significant impact for the treatment of this disease. We also sought to evaluate the association of SNPs in key oxidant and antioxidant enzymes with increased risk and survival in epithelial ovarian cancer. Individuals (n = 143) recruited were divided into controls, (n = 94): healthy volunteers, (n = 18), high-risk BRCA1/2 negative (n = 53), high-risk BRCA1/2 positive (n = 23) and ovarian cancer cases (n = 49). This study indicates a strong association with the catalase SNP and survival of ovarian cancer patients, and thus may serve as a prognosticator (10).

9. **Fletcher NM**, Belotte J, Saed MG, Memaj I, Diamond MP, Morris RT, Saed GM. Specific point mutations in key redox enzymes are associated with chemoresistance in epithelial ovarian cancer. Free Radic Biol Med. 2017 Jan;102:122-132. PMID: 27890641.

10. *Belotte J, ***Fletcher NM**, Saed MG, Abusamaan MS, Dyson G, Diamond MP, et al. A Single Nucleotide Polymorphism in Catalase Is Strongly Associated with Ovarian Cancer Survival. PloS one. 2015;10:e0135739. PMID: 26301412; PMCID: PMC4547699. **Indicates co-authorship.*

3) *The discovery of the adhesion phenotype.* An additional significant contribution is the discovery of the "adhesion pheontype." We have shown that fibroblast cultures established from fresh adhesion tissues manifest a unique phenotype that resembles myofibroblasts, which we have termed the "adhesion phenotype." Specifically, the adhesion phenotype is characterized by increased α-smooth muscle actin, increased transforming growth factors and cytokines and extracellular matrix production, decreased extracellular matrix turnover via effects on matrix-degrading enzymes and their inhibitors, as well as decreased apoptosis (11). Remarkably, hypoxia causes normal peritoneal fibroblasts to irreversibly acquire the adhesion phenotype (12). The generated superoxide ($O_2^{\bullet-}$) has been previously demonstrated to be a key player in formation of the adhesion phenotype, and hypoxia induced an oxidative stress environment that favors the development of this phenotype (12). Adhesion fibroblasts manifest decreased superoxide dismutase (SOD3), caspase-3 activity, nitrate/nitrite (NO_3^-/ NO_2^-), as well as increased NADPH oxidase, S-nitrosylation of caspase-3, and lipid peroxidation, as compared to normal peritoneal fibroblasts (11). Hypoxia simultaneously increases extracellular matrix production and decreases turnover via effects on matrix-degrading enzymes and their inhibitors leading to the manifestation of an adhesion phenotype. The mechanism by which hypoxia induces the development of the adhesion phenotype is believed to involve endogenous production of $O_2^{\bullet-}$. Indeed, scavenging $O_2^{\bullet-}$, by SOD, restores the adhesion phenotype markers, transforming growth factor-β1 and type I collagen, levels in adhesion fibroblasts to levels observed in normal peritoneal fibroblasts (12). Furthermore, scavenging $O_2^{\bullet-}$ during hypoxia exposure protected against the development of the adhesion phenotype. Normal peritoneal fibroblasts treated with SOD combined with hypoxia prevented acquisition of the adhesion phenotype (12). Collectively, we have unequivocal evidence to support hypoxia as a result of tissue injury to be the cause of several profibrotic disorders, including postoperative adhesion.

11. Awonuga AO, Belotte J, Abuanzeh S, **Fletcher NM**, Diamond MP, Saed GM. Advances in the Pathogenesis of Adhesion Development: The Role of Oxidative Stress. Reprod Sci. 2014 Jul;21(7):823-836. Review. PMID: 24520085; PMCID: PMC4107571.

12. **Fletcher NM**, Jiang ZL, Diamond MP, Abu-Soud HM, Saed GM. Hypoxia-generated superoxide induces the development of the adhesion phenotype. Free Radic Biol Med. 2008;45:530-6. PMID: 18538674; PMCID: PMC2574925.

4) *Ex vivo postoperative adhesion model.* Recently, we have established an *ex vivo* model that provides important information on the pathophysiology of adhesion development. First, it demonstrates that grossly visible adhesive bands can form within 24 hours, and that at this early time point it can already be dense and opaque (as opposed to filmy and transparent) (13). Secondly, consistent with clinical teaching, the placement of a blood clot between peritoneal surfaces incited greater incidence, tissue bloc surface area involvement, and tenacity of the adhesion band(s) that developed (13). Thus, these findings are consistent with the concept of attempting to achieve hemostasis to minimize adhesion development. Thirdly, the incidence, surface area of involvement, and tenacity of the adhesions that developed were greater following tissue traumatization (simulating tissue injury such as grasping with manipulation during the surgical procedure). Overall, this *ex vivo* peritoneal strip adhesion model provides a potentially valuable approach to examine efficacy of postoperative adhesion adjuvants as well as the study of the molecular biologic processes underlying development of adhesions (13).

13. Saed GM, **Fletcher NM**, Diamond MP. The Creation of a Model for Ex Vivo Development of Postoperative Adhesions. Reproductive Science. Reprod Sci. 2016 May;23(5):610-2. PMID: 26408397.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/1xiXzcfGjF7Q5/bibliography/46200971/public/?sort=date&direction=ascending

D. Research Support

Previous Research Support

Prevent Cancer Foundation Nicole M. King (PI)
01/16-01/18
Postdoctoral Fellowship Grant
Novel biomarkers for early detection of ovarian cancer
The major goal of this project is to determine whether myeloperoxidase and free iron can be utilized as biomarkers for the early detection of ovarian cancer alone and in conjunction with currently available screening tools.
Role: PI

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Robert T. Morris, M.D.

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Professor, Program Director

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Saint John's University Collegeville, Minnesota	Bachelor of Science	1984	Chemistry
University of Minnesota, Minneapolis, Minnesota	Doctor of Medicine	1988	Doctor of Medicine
University of Michigan, Ann Arbor, Michigan		1990-1991	Cellular and Molecular Biology Doctorate Program
Wayne State University, Detroit, Michigan		1991-1992	Cancer Biology Doctorate Program
Wayne State University/Detroit Medical Center, Detroit, Michigan	Residency	1994	Obstetrics and Gynecology
The University of Texas MD Anderson Cancer Center	Fellowship	1996	Gynecologic Oncology

A. Personal Statement

I am a professor of gynecologic oncology, and my responsibilities include caring for patients with gynecologic malignancies, training residents and fellows, and advancing the knowledge in my field through research. I am well suited for participation and leadership in the research program because of my training, my research experience and production, as well as my contribution to education. In addition to two years of basic science post-graduate education at the University of Michigan and Wayne State University, I graduated from a very rigorous fellowship at the MD Anderson Cancer Center. I have been an institutional PI for over 30 studies at Wayne State University, and have been the PI on two investigator initiated multi-institutional clinical trials (1,2) As the director of Gynecologic Oncology, I am committed to overseeing the scientific efforts, the educational mission, and the medical management of patients with gynecologic malignancies. My research efforts include mentoring and directly participating with fellows in bench research as well as direct development, supervision, and participation in clinical research. In recognition of these qualifications and activities I have been a member of the Ovary Committee of the Gynecologic Oncology Group as well as contributed invited editorials from impactful journals (3).

1. Morris RT, Alvarez RD, Andrews S, Malone J, Bryant CS, Heilbrun LK, Schimp V, Munkarah AR. Topotecan weekly bolus chemotherapy for relapsed platinum-sensitive ovarian and peritoneal cancers. Gynecol Oncol. 2008. 109. 346-352.
2. Morris RT, Joyrich RN, Naumann RW, Shah NP, Maurer AH, Strauss HW, Uszler JM, Symanowski JT, Ellis PR, Harb WA. Phase II study of treatment of advanced ovarian cancer with folate-receptor-targeted

therapeutic (vintafolide) and companion SPECT-based imaging agent (99m Tc-etarforatide). Ann Oncol. 2014 Apr;25(4):852- 8.
3. Morris RT, Monk BJ. Ovarian cancer: relevant therapy, not timing, is paramount. Lancet. 2010 Oct 2;376(9747):1120-2.

B. Positions and Honors

Positions and Employment

2005 – 2008	Wayne State University School of Medicine, Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Interim Director, Detroit, Michigan
2005 – Present	Wayne State University School of Medicine, Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Director, Fellowship Program, Detroit, Michigan
2006 – 2009	Wayne State University School of Medicine, Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Director, Detroit, Michigan
2009 – Present	Wayne State University School of Medicine, Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Interim Director, Detroit, Michigan
2009 – Present	Karmanos Cancer Institute, Wayne State University School of Medicine, Gynecologic Oncology Multidisciplinary Team Leader, Detroit, Michigan
2016 – Present	Wayne State University School of Medicine, Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Maria E. Brasza Endowed Chair in Gynecologic Oncology

Honors

1988	Minnesota Medical Foundation Scholarship
1990 – 1991	National Institute of Health Human Genetic Training Grant
1992	Felix Rutledge Fellowship, MD Anderson Cancer Center, Houston, Texas
1992 – 1994	Holden Research Fellowship Grant
1993 – 1994	Stevenson Award for Academic Excellence, Wayne State University, Detroit, Michigan
1994	Wayne State University Fellows and Residents Outstanding Research Award, Detroit, Michigan
2000	Wayne State University School of Medicine Excellence in Teaching Award, Detroit, Michigan
2001, 2002, 2016	American College of Obstetricians and Gynecologists Council on Resident Education in Obstetrics and Gynecology National Faculty Award
2004, 2016	St. John Hospital and Medical Center Instructor of the Year
2006 - 2016	Detroit's Top Docs: Hour Detroit Magazine
2014	St. John's University Alumni Achievement Award

C. Contribution to Science

1) Folate targeted imaging in ovarian cancer: Ovarian cancer is a highly lethal malignancy. Although it initially responds to cytotoxic chemotherapy, it ultimately recurs and becomes chemoresistant. Predicting response to subsequent therapy has been challenging. Folate receptors are over- expressed on a large proportion of ovarian cancer. In a large multi-institutional phase 2 study, I investigated vintafolide folate targeted imaging prior to treatment with a folate targeting agent (EC45). This study demonstrated that those patients with folate positive imaging responded better to the therapy. This study has been part of the basis for our institutions commitment to folate targeted therapy and imaging. Morris RT, Joyrich RN, Naumann RW, Shah NP, Maurer AH, Strauss HW, Uszler JM, Symanowski JT, Ellis PR, Harb WA. Phase II study of treatment of advanced ovarian cancer with folate-receptor-targeted therapeutic (vintafolide) and companion SPECT-based imaging agent (99m Tc-etarforatide). Ann Oncol. 2014 Apr;25(4):852-8.

2) Chemotherapy administration dosing and scheduling: Although drug dosage is rigorously established through preclinical and phase 1 studies, the optimal schedule is often determined on a more theoretic basis. Topotecan is a topoisomerase inhibitor indicated in the treatment of ovarian cancer. It was developed as a 5 day infusion. Due to to toxicity and early clinical observations that weekly administration of other cell cycle specific agents may maintain or improve activity and diminish toxicity I developed a multi-institutional trial evaluating another dosing schedule of topotecan. This study demonstrated lower than expected toxicity while still demonstrating anticancer activity. These data contributed to the basis for 2 subsequent phase 3 cooperative group trials. Morris RT, Alvarez RD, Andrews S, Malone J, Bryant CS, Heilbrun LK, Schimp V, Munkarah AR. Topotecan weekly bolus chemotherapy for relapsed platinum-sensitive ovarian and peritoneal cancers. Gynecol Oncol. 2008. 109. 346-352.

3) Surgery: Ovarian cancer is the third leading cause of cancer deaths among women in the united staes. Due to its late presentation and aggressive clinical behavior, aggressive surgery and chemotherapy are used in nearly all patients. I have participated clinical trials defining the role of aggressive surgery in the management of ovarian cancer and assisted in manuscript preparation of the study. Overall the study found that volume of residual disease following surgery for recurrent disease was a predictive factor for disease free survival. Currently the goal of secondary surgery is to attain the level of no residual disease. Disease extent at secondary cytoreductive surgery is predictive of progression-free and overall survival in advanced stage ovarian cancer: An NRG Oncology/Gynecologic Oncology Group study. Rose PG, Java JJ, Morgan MA, Alvarez-Secord A, Kesterson JP, Stehman FB, Warshal DP, Creasman WT, Hanjani P, Morris RT, Copeland LJ. Gynecol Oncol. 2016 Dec;143(3):511-515. doi: 10.1016/j.ygyno.2016.09.005.

4) Mentoring: Chemotherapy cures some patients with ovarian cancer. Although some develop life- long toxicities such as neuropathy or cognitive impairment, some will ultimately develop secondary malignancies such as leukemia or myelodysplastic syndrome. One of my fellows questioned me as to the risk of such secondary cancers, and because the existing data was irrelevant a largescale epidemiologic review of the SEER database was performed. The development, supervision, and authorship of this study was by my fellow Dr A Vay under my mentorship. I include this as my contribution as an example of sheparding trainees through the process of inquiry, investigation,and reporting. Vay A, Kumar S, Seward S, Semaan A, Schiffer CA, Munkarah AR, Morris RT. Therapy-related myeloid leukemia after treatment for epithelial ovarian carcinoma: An epidemiological analysis. Gynecol Oncol. 2011 Aug 18

Human primary normal ovarian epith
From [Cell Biologics] CATAL
Media : Epithelial cell Medium/w Kit

Cell dose Curve

1.Cell-dose curve

Prepare cell-dose curve and time zero samples. Measure absorbance
nm

5,

Cell-dose curve:

1). On day 0, prepare a cell-dose curve by using the stock (1
cells/ml in 1 X DMEM/10% FBS medium).

2). Prepare Blank (1X DMEM/10% FBS, no cells) and seven s
dilutions of cells (2-fold) in separate 1.5 mL centrifuge tubes
1X DMEM/10% FBS as diluent.

Perform Serial Dilution by...

- a. Using an 8 channel multi-pipette, add 150 µl of m
each well of a 96 well microplate.
- b. Add 150 µl of $5.34x10^5$ cells /ml ($80x10^3$ cells) solu
the wells of first duplicate row.
- c. Take 150 µl from the first duplicate row, add it to
well and mix. The process is repeated as blow pictur
- Reserve the final well for the negative control (Blank
well should contain media only (no cells).
- Also make a Test Cells(30K cells/well)
- Total volume will be 150 µL of each mixture into a se
well of a 96-well clear plate.

3). Add 35 µL of 1X DMEM/10% FBS and 15 µL of WST Work
Solution into each well (Blank, Cell Standard Curve and Test
4) incubate at 37°C in an incubator for 4 hours.
5). Measure absorbance using a microtiter plate reader at 4

PLATE TEMPLATE

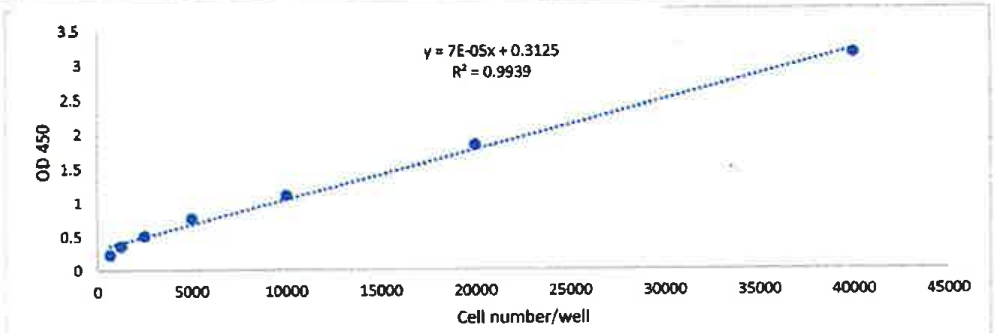
	1	2	3	4	5	6	7	8	9	10	11	12
A	40K											
B	20K		30K									
C	10K											
D	5K											
E	2.5K											
F	1.25K											
G	0.625K											
H	Blank											

STD

Talcum powder (J&J baby pow
30027477 , lot # 137

Results:

Standard with PBS as Diluent							
	Cell Number	OD1	OD2	Average	Corrected 1	Corrected 2	Average
A	40000	3.9603	4.1406		3.05695	3.23725	3.1471
B	20000	2.7654	2.6965		1.86205	1.79315	1.8276
C	10000	2.0421	1.966		1.13875	1.06265	1.1007
D	5000	1.6562	1.6711		0.75285	0.76775	0.7603
E	2500	1.3806	1.4131		0.47725	0.50975	0.50975
F	1250	1.2585	1.2606		0.35515	0.35725	0.35725
G	675	1.1542	1.1118		0.25085	0.20845	0.22965
Blank	0	0.9156	0.8911	0.90335	0.9156	0.8911	0.90335
Test sample	31904	3.3764	3.5218		2.47305	2.61845	2.54575



Dissolve with PBS @ 50m
" " " @ 10 m

Sonicate 3 times for 1 min ea
Sonic Dismembrator (Fisher Sc

For 100 mm dishes will

100 µl of 50 mg/ml to gi
100 µl of 10 mg/ml to gi
100 µl of PBS as co

Normal Ovarian Epithelial Cells (Lot 3)
Cell Biologics

Cells were seeded @ a density of 30,000 as follows:

Blank (medium)
Control (medium + 30K cells)
Negative Control (medium + 30K cells + PBS)
Exp1 (100ug/ml Talc + 30K cells)
Exp2 (500ug/ml Talc + 30K cells)
Exp3 (100ug/ml TiO_2 + 30K cells)
Exp 4 (500 ug/ml TiO_2 + 30K cells)

1) **Agarose powder**

- To make a 1.2% solution of Agarose powder: add 20 m Agarose into the Agarose Powder bottle
- Slightly open the bottle cap and place it on a heat bloc powder is entirely dissolved, gently shake the bottle to
- Move that bottle to a water bath of 37°C for 30 minut equilibrium.
- The 1.2% of agarose solution that isn't used can be sto conditions.

2) **Preparation of DMEM solution (1 x DMEM plus 10 FB**

- DMEM (10x)
- Water
- FBS (Hyclone Cat # SH30396.03, Lot# AD19958293)

Store at 4°C, but before using warm it up to 37°C in a wat

3) **Preparation of WST reagent and electro coupling sol**

- Add 1.8 mL of Electro Coupling Solution to the WST Re Working Solution.
- For short term storage place 4°C for 6 months, for lon and protect it from light.

4) **Staining solution**

- Ready to use as supplied

Assay Procedure

1) Preparation of Base Agarose layer

-Prepare 75 µL per well of base agarose mix as follows:

1 Well:

Component	Volume (µL)
1.2% Agarose solution	37.5
DMEM Solution (10X)	7.5
FBS	7.5
dH_2O	22.5

12 Wells for 5 samples:

Component	Volume (µL)*
1.2% Agarose solution	450
DMEM Solution (10X)	90
FBS	90
dH_2O	270

*To account for error/extras we multiplied by 1.17

- Add 75 µL of the base agarose mix into required wells in the 96 well clear bottom tissue culture plate.
- Keep the plate at 4°C for 15 minutes to solidify the agarose.

2) Preparation of top Agarose layer with cells

- Use 30000 cells per well (20 µL of 1.5 x 10^6 cells/mL)
- Preparation of 75 µL/well mix of top Agarose as follows:

Component	1 well (µL)	12 wells ((µL) *
1.2% Agarose solution	25.0	300
DMEM Solution (10X)	5.5	66
FBS	5.5	66
Cells in 1XDMEM/10% FBS	20	240
dH_2O	19	228

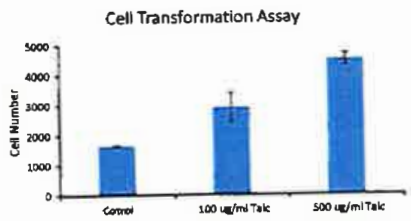
*To account for error all of the 12 wells will be multiplied by 1.12

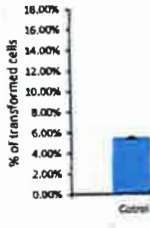
- Mix it in by using a pipette
- Add 75 µL of agarose-cell mix to wells to the base Agarose 96 well plate (step 1).
- Place the plate at 4°C for 10 minutes to solidify the layer.
- Then bring the plate back down to room temperature by placing it in the tissue culture hood for 10 minutes.
- Add 100 µL of 1X DMEM/10% FBS medium with and without talcum powder and incubate at 37°C for 7 days.
- Remove the upper medium on the top agarose layer by pipetting.
- Add 35 µL of 1X DMEM/10% FBS and 15 µL of WST Working Solution into each well.
- Incubate for 4 hours at 37°C and then using a microtiter plate reader measure the absorbance at 450 nm.

Results:

8/5/2019 Seeded 30K/well Normal Ovarian Epithelial cells
8/12/2019
Temperature(°C)
24.8
450 OD

Treatment (ug/ml)	OD 1	OD 2	Average	Corr OD1	Corr OD2	cell number 1	cell number 2	Average
Blank	0.98	0.94	0.96					
Control	1.38	1.39	1.38	0.43	0.43	1615.71	1671.43	1643.5
100 ug/ml Talc	1.44	1.51		0.48	0.55	2441.43	3400.00	2920.7
500 ug/ml Talc	1.57	1.60		0.61	0.64	4314.29	4708.57	4511.4



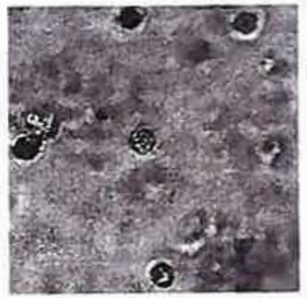


Need to run TiO_2

Cells were successfully transfor

Normal Ovarian Cells

Control



100 ug/ml Talc

8.12.2019

- human normal ovarian epithelial cells [Cell Biologics]
- human primary normal peritoneal fibroblasts from our lab.

- Seeded cells to density of 30K as follows:

Blank	only Medium
Control	Medium + 30K cells
Negative Control	Medium + 30K cells + PBS
Exp1	100 µg/ml Talcum powder + 30K cells
Exp2	500 µg/ml " " "
Exp3	100 µg/ml TiO_2 " "
Exp4	500 µg/ml TiO_2 " "

Treat cells for 72hrs

perform transformation assay exactly as previous experiment. (start on 8.15.2019)

Results: 8/22/2019

8.12.2019 Seeded 30K/well Normal Ovarian Epithelial cells
450 OD
8/22/19

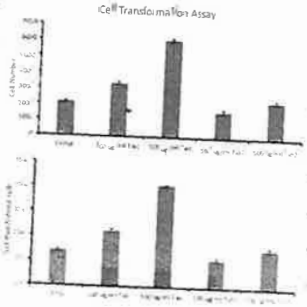
Temperature(°C)	24.7	1	2	3	4	5	6	7
		0.0332	0.0328	0.0344	0.0322	0.041	0.0413	0.0395
		0.0315	0.0378	0.949	0.9286	0.1422	0.0391	0.0351
		0.0377	0.0384			0.0384	0.0384	0.0369
		0.041	0.0396			0.07721	0.0469	0.0388
		0.0353	0.0387					0.0379
		0.0361	0.0411					0.0371
		0.041	0.0399					0.0387
		0.0332	0.0396	0.0386	0.0379	0.0379	0.0394	0.0379

Treatment (mg/ml)	OD 1	OD 2	Average	Corr OD 1	Corr OD 2	cell number 1	cell number 2	Average	SD	Cell Survival Ratio(%)	Cell Survival Ratio(%)	Average	SD
Blank	0.949	0.9386	0.9438										
Control	1.4613	1.4711	1.4662	0.5175	0.5273	2928.571	3068.571	2998.57	70.00	100%	30%	30%	0%
Negative Control	1.4813	1.4104	1.445	0.4575	0.4666	2671.429	2701.429	2136.43	65.00	7%	7%	7%	0%
100 ug/ml Talc	1.4807	1.4373		0.5368	0.5535	3203.714	3442.857	3324.29	118.57	11%	11%	11%	0%
500 ug/ml Talc	1.6788	1.6803		0.7351	0.7455	6037.143	6185.714	6111.43	74.29	20%	21%	20%	0%
100 ug/ml TiO2	1.3839	1.3632		0.4401	0.4194	1822.857	1527.143	1675.00	147.86	6%	5%	6%	0%
500 ug/ml TiO2	1.4303	1.4105		0.4865	0.4667	2485.714	2202.857	2344.29	141.43	8%	7%	8%	0%

Results: 8/22/2019

100 µg/ml Talcum powder

100 µg/ml TiO_2



500 µg/ml Talcum powder

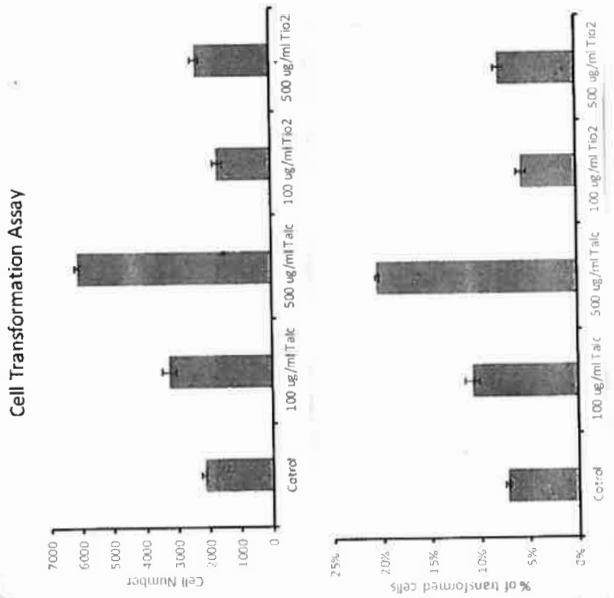
Normal ovarian epithelial cells

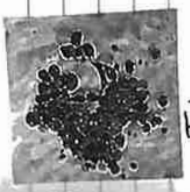
Normal peritoneal Fibroblasts

100 µg/ml Talcum powder

500 µg/ml Talcum Powder

100 µg/ml TiO_2





Talc
Talc 100ug/ml (Duplicates)

8.29.2019 Seeded 30K/well Normal Ovarian Epithelial cells

450 OD
9/2/19

Temperature(°C)	1	2	3	4	5	6	7
24.8	0.0402	0.0328	0.0247	0.0322	0.0407	0.041	0.0391
	0.0323	0.0386	0.9481	0.9375	0.1412	0.0371	0.039
	0.0389	0.0386	1.4605	1.4751	0.0395	0.0376	0.0373
	0.0411	0.0385	1.4026	1.4125	0.0374	0.0474	0.0392
	0.0345	0.0383	1.4666	1.4973	1.3839	1.3632	0.0383
	0.035	0.04	1.6793	1.6884	1.4303	1.4105	0.0569
	0.0407	0.0387	0.0377	0.0377	0.0382	0.038	0.0388
	0.0389	0.0386	0.0383	0.0381	0.0382	0.0396	0.0383

8.23.19

Repeat previous experiment to confirm results

Seed cells exactly as described for previous experiment (8.12.19).

Treatment (ug/ml)	OD 1	OD 2	Average	Corr OD1	Corr OD2	cell number 1	cell number 2	Average
Blank	0.9481	0.9375	0.9428					
Control	1.4605	1.4751	1.4678	0.5177	0.5323	2931.42857	3140	3035
Negtive Cotrol	1.4026	1.4125	1.40755	0.4598	0.4697	2104.28571	2245.71429	2175
100 ug/ml Talc	1.4666	1.4973		0.5238	0.5545	3018.57143	3457.14286	3237
500 ug/ml Talc	1.6793	1.6884		0.7365	0.7456	6057.14286	6187.14286	6122
100 ug/ml Tio2	1.3839	1.3632		0.4411	0.4204	1837.14286	1541.42857	1689
500 ug/ml Tio2	1.4303	1.4105		0.4875	0.4677	2500	2217.14286	2358

9/3/2019

~~HOSEpic~~ Cells were seeded @ density of 30K cells per well as follows

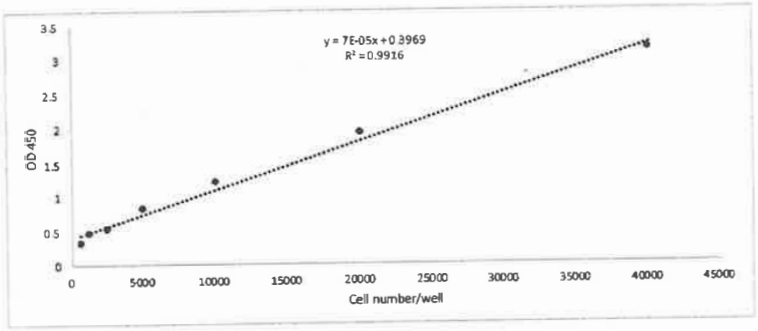
Blank	exactly as described in exp.
Untreated	dated 8/2/2019
TiO_2 100ug/ml	
$Ti O_2$ 500ug/ml	In triplicate
Talc 100ug/ml	
Talc 500ug/ml	Treat cells with Talcum powder for 72hrs.

Cell dose curve was performed exactly as previously described on 8/1/2019 using HOSEpic cells

Calculate cell number

Standard with PBS as Diluent

	Cell Number	OD1	OD2	Average	Corrected 1	Corrected 2	Average	
A	40000	3.833	3.9749		3.05525	3.19715	3.1262	
B	20000	2.6825	2.7006		1.90475	1.92285	1.9138	
C	10000	1.9032	2.0532		1.12545	1.27545	1.20045	
D	5000	1.5773	1.6114		0.79955	0.83365	0.8166	
E	2500	1.2863	1.3226		0.50855	0.54485	0.5267	
F	1250	1.1987	1.2711		0.42095	0.49335	0.45715	
G	675	1.0871	1.1118		0.30935	0.33405	0.3217	
Blank	0	0.7744	0.7811	0.77775	-0.00335	0.00335	5.551E-17	
sample test(30000 cells)		3.1866	3.2112		2.40885	2.43345	2.42115	30124



9/27/2019

Seeded 30000/well HOSEpiC cells
9/27/19
Temperature(°C) 25.8
450nm OD

HOSEpiC	Temp(°C)	1	2	3	4	5	6	7	8	9	10	11	12
	25.8	0	0									0	0
		0	0									0	0
		0	0	0.8871	0.7866	1.332	1.3544	1.2561	1.2782	1.3307	1.3622	0	0
		0	0	1.811	1.7923	2.3021	2.3772					0	0
		0	0	0.7941	0.8553	1.2784	1.2881	1.3113	1.3411	1.2259	1.2733	0	0
		0	0	1.9778	2.111	2.4337	2.3888					0	0
		0	0	0.8343	0.8122	1.2591	1.2673	1.244	1.2611	1.3112	1.323	0	0
		0	0	2.0091	2.1155	2.3317	2.3877					0	0

Treatment	OD1	OD2	Avg	Corr OD1	Corr OD2	Cell #1	Cell #2	Avg cell #	SD	Survival Ratio 1	Survival Ratio 2	Avg	SD
Blank	0.89	0.79	0.84										
Untreated	1.33	1.35	1.34	0.50	0.52	1403.57	1723.57	1563.57	160.00	5%	6%	5%	1%
Neg ctrl TiO2 100 ug/ml	1.26	1.28	1.27	0.42	0.44	319.29	635.00	477.14	157.86	1%	2%	2%	1%
Neg ctrl TiO2 500 ug/ml	1.33	1.36	1.35	0.49	0.53	1385.00	1835.00	1610.00	225.00	5%	6%	5%	1%
Talc 100ug/ml	1.81	1.79	1.80	0.97	0.96	8246.43	7979.29	8112.86	133.57	27%	27%	27%	0%
Talc 500ug/ml	2.30	2.38	2.34	1.47	1.54	15262.14	16335.00	15798.57	536.43	51%	54%	53%	2%

Treatment	OD1	OD2	Avg	Corr OD1	Corr OD2	Cell #1	Cell #2	Avg cell #	SD	Survival Ratio 1	Survival Ratio 2	Avg	SD
Blank	0.79	0.86	0.82										
Untreated	1.28	1.29	1.28	0.45	0.48	811.43	950.00	880.71	69.29	3%	3%	3%	0%
Neg ctrl TiO2 100 ug/ml	1.31	1.34	1.33	0.49	0.52	1281.43	1707.14	1494.29	212.86	4%	6%	5%	1%
Neg ctrl TiO2 500 ug/ml	1.23	1.27	1.25	0.40	0.45	61.43	738.57	400.00	338.57	0%	2%	1%	1%
Talc 100ug/ml	1.98	2.11	2.04	1.15	1.29	10802.86	12705.71	11754.29	951.43	36%	42%	39%	3%
Talc 500ug/ml	2.43	2.39	2.41	1.61	1.56	17315.71	16674.29	16995.00	320.71	58%	56%	57%	1%

Treatment	OD1	OD2	Avg	Corr OD1	Corr OD2	Cell #1	Cell #2	Avg cell #	SD	Survival Ratio 1	Survival Ratio 2	Avg	SD
Blank	0.83	0.81	0.82										
Untreated	1.26	1.27	1.26	0.44	0.44	556.43	673.57	615.00	58.57	2%	2%	2%	0%
Neg ctrl TiO2 100 ug/ml	1.24	1.26	1.25	0.42	0.44	340.71	585.00	462.86	122.14	1%	2%	2%	0%
Neg ctrl TiO2 500 ug/ml	1.31	1.32	1.32	0.49	0.50	1300.71	1469.29	1385.00	84.29	4%	5%	5%	0%
Talc 100ug/ml	2.01	2.12	2.06	1.19	1.29	11270.71	12790.71	12030.71	760.00	38%	43%	40%	3%
Talc 500ug/ml	2.33	2.39	2.36	1.51	1.56	15879.29	16679.29	16279.29	400.00	53%	56%	54%	1%

100 ug/ml Talc | 500 ug/ml Talc | 100 ug/ml $Ti O_2$ | NO treatment control.

9/27/2019

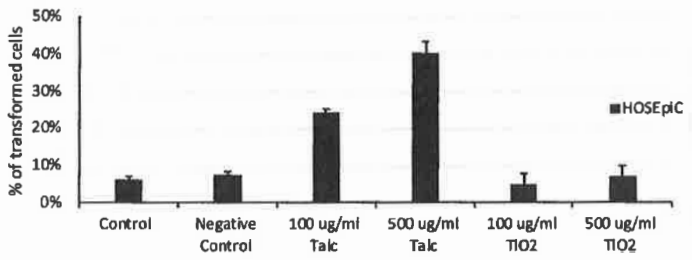
HOSEpic cells were seeded @ 30K density per well as follows (exactly as described on 9/3/2019)

Using the cell dose curve performed on 9/3/2019 we reported the EXP.

9/27/19 Seeded 30000/well HOSEpiC cells
10/4/19
Temperature(°C) 25.8
450nm OD

HOSEpiC cells	Temp(°C)	1	2	3	4	5	6	7	8	9	10	11	12
	25.8	0.05	0.06	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
		0.04	0.05	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
		0.04	0.04	0.98	0.97	1.42	1.46	1.37	1.41	1.41	1.45	0.04	0.05
		0.04	0.04	1.74	1.85	2.19	2.08	0.05	0.04	0.04	0.04	0.04	0.05
		0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.07	0.04	0.05	0.05	0.04
		0.05	0.05	0.03	0.04	0.04	0.05	0.06	0.04	0.05	0.04	0.04	0.05
		0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.05
		0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

Treatment	OD1	OD2	Avg	Corr OD1	Corr OD2	Cell #1	Cell #2	Avg cell #	SD	Survival Ratio 1	Survival Ratio 2	Avg	SD
Blank	0.98	0.97	0.98										
Untreated	1.42	1.46	1.44	0.45	0.48	1899.29	2459.29	2179.29	280.00	6%	8%	7%	1%
Neg ctrl TIO2 100 ug/ml	1.37	1.41	1.39	0.39	0.43	1145.00	1673.57	1409.29	264.29	4%	6%	5%	1%
Neg ctrl TIO2 500 ug/ml	1.41	1.45	1.43	0.43	0.48	1727.86	2325.00	2026.43	298.57	6%	8%	7%	1%
Talc 100ug/ml	1.74	1.85	1.80	0.76	0.87	6457.86	8012.14	7235.00	777.14	22%	27%	24%	3%
Talc 500ug/ml	2.19	2.08	2.14	1.21	1.10	12829.29	11316.43	12072.86	756.43	43%	38%	40%	3%



12.8.2020

Two normal ovarian epithelial cell lines
→ Human primary normal ovarian epithelial cells (Cell Biologics)
→ Human ovarian ~~Sexirface~~ epithelial cells HOSEpiC (~~Scien(a)~~)

- Cells were grown in 100mm plate and treated with 100 µg/ml talcum powder see methods described on 8/2/2019
- treatment was performed for 72hrs in 37°C
- Cells were given to WSU, Department of Pathology to perform Immunohistochemistry

NOEC DAPI control
NOEC Ki67 =
NOEC P53 =
NOEC DAPI talc (100ug/ml) 72hrs.
NOEC Ki67 = = =
NOEC P53 = = =
HOSEpiC DAPI control
HOSEpiC Ki67 =
HOSEpiC P53 =
HOSEpiC DAPI talc (100ug/ml) 72hrs
HOSEpiC Ki67 = = =
HOSEpiC P53 = = =

IHC staining and scoring

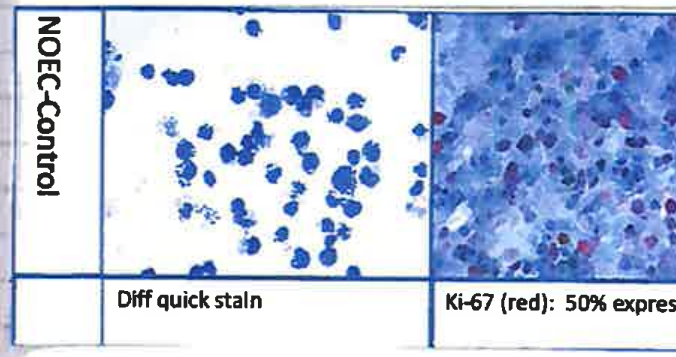
The IHC panel consisted of antibodies against p53 and Ki-67. The primary antibodies, suppliers, and staining conditions are listed in Table 1.

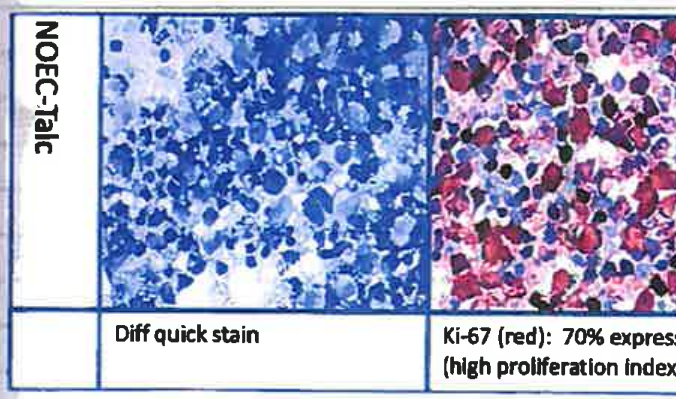
Table 1
Antibodies and staining conditions for immunohistochemistry

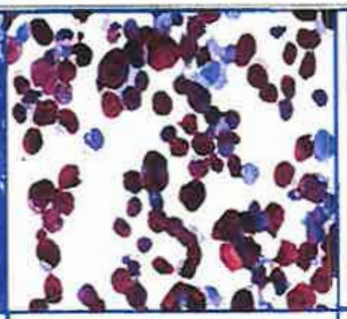
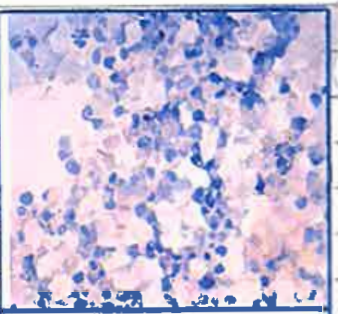
Antibody	Clone	Source	Detection system	Dilution
P53	DO-7	Ventana	Ventana ultraView DAB	1:500
Ki-67	Mib1	Ventana	Ventana ultraView DAB	1:2000

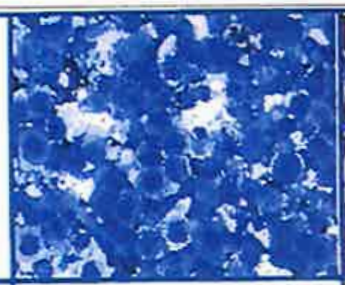
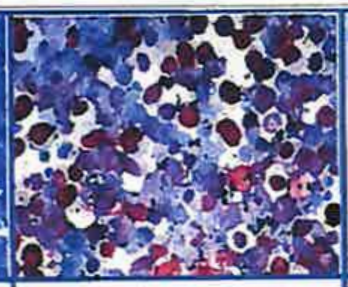
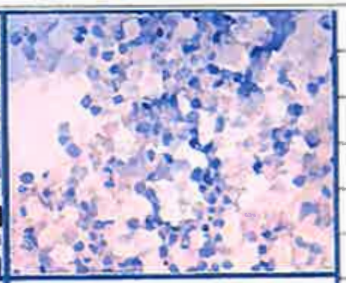
Cytospin slides prepared from cell line cultures and stained (image1) using immunoperoxidase labeling performed with the automated XT iVIEW DAB V.1 procedure on the Ultra BenchMark XT IHC/ISH Staining Module, Ventana with anti-p53 (clone DO-7 prediluted, Ventana). Antigen retrieval was carried out with CC1, pH 8.0 (Ventana). Sections were incubated with primary antibodies for 36 min at 37°C. All slides were reviewed by two pathologists (RA and AA). Cases with discordant Ki-67 estimated results underwent a consensus review at a double-headed microscope. Diffuse "in-block" nuclear staining or complete negative staining with p53 was considered a positive reaction indicating mutated p53 status. Focal nuclear staining is consistent with "wild type" p53 and considered negative. The Proliferation Index (PI) was assessed qualitatively using Ki-67 stained slides and classified as high PI (>50% positive cells) or low PI (<50% positive cell) [1].

Refrence
[1] MAhAdevAPPA, AShA, Shruthi Mysore Krishna, and Manjunath Gubbanna Vimala. "Diagnostic and prognostic significance of Ki-67 immunohistochemical expression in surface epithelial ovarian carcinoma." Journal of Clinical and Diagnostic Research: JCDR 11.2 (2017): EC08.





HOSPIC-Control			
	Diff quick stain	Ki-67 (red): 70% expression (high proliferation index)	P53 (red): In-block staining (mutated p53)

HOSPIC-Talc			
	Diff quick stain	Ki-67 (red): 50% expression	P53 (red): focal staining with variable intensity (wild type

Invoice

Date: 07/14/2020
Invoice No.: 10063
Due Date: 07/24/2020

DS Biotech, LLC
1665 Dell Rose
Bloomfield Hills, MI 48302
Ghassan Saed

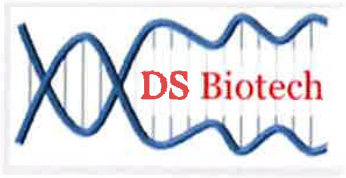
Bill To:
Beasley Allen
Beasley Allen
218 Commerce St
Montgomery, AL 36104

Qty	Item	Description	Unit Price	Total
65	Consulting	Consulting	$600.00	$39,000.00

Total **$39,000.00**
Balance Due **$39,000.00**

Please contact us for more information about payment options.

Thank you for your business.



DS Biotech, LLC

1665 Dell Rose
Bloomfield Hills, MI 48302
248-894-1474
gsaed@dsbiotech.net

INVOICE
INV0004

DATE
Jan 5, 2021

DUE
On Receipt

BALANCE DUE
USD $27,600.00

BILL TO

Beasley Allen

218 Commerce st
Montgomery, Al
36104
3345465435
leigh.odell@BeasleyAllen.com

DESCRIPTION	RATE	QTY	AMOUNT
Consultation	$600.00	46	$27,600.00

SUBTOTAL	$27,600.00
TAX (0%)	$0.00
TOTAL	$27,600.00
BALANCE DUE	**USD $27,600.00**

From: McKay, Samantha (HC/SC) samantha.mckay@canada.ca
Subject: RE: A-2018-001795
Date: June 24, 2020 at 11:00 AM
To: Ghassan Saed gsaed@med.wayne.edu

SM

Hello,
The request is for records in relation to the Draft Screening Assessment – Talc and Health Canada's communication with you regarding it. The records pertaining to yourself specifically is a few emails and a published document (31 pages in total).
I would need you to review the records and identify any information you feel should not be released to the public.

Regards,

Samantha McKay
Consultant
Access to Information & Privacy
Health Canada and Public Health Agency of Canada / Government of Canada
samantha.mckay@canada.ca

Samantha McKay
Consultante
Accès à l'information et de la protection des renseignements personnels Santé Canada et Agence de la santé publique du Canada / Gouvernement du Canada
samantha.mckay@canada.ca

From: Ghassan Saed <gsaed@med.wayne.edu>
Sent: 2020-06-24 10:54 AM
To: McKay, Samantha (HC/SC) <samantha.mckay@canada.ca>
Subject: Re: A-2018-001795

What kind of information they are requesting?

Best Regards
Dr. Ghassan Saed, Associate Professor
Department of OB/GYN
Wayne State University Medical School
Detroit, MI

> On Jun 24, 2020, at 2:34 AM, McKay, Samantha (HC/SC) <samantha.mckay@canada.ca> wrote:
>
> Hello,

Hello,
I hope this email finds you well. I am processing the above noted ATIP request and have identified some records that pertain to you, I will need to consult with you prior to releasing them to the requester.

As we are currently unable to utilise mail/courier services, I will need to send them via email. If you could confirm receipt of this email in a timely manner it would be greatly appreciated.

Thank you in advance for your assistance,

Samantha McKay
Consultant
Access to Information & Privacy
Health Canada and Public Health Agency of Canada / Government of Canada
samantha.mckay@canada.ca

Samantha McKay
Consultante
Accès à l'information et de la protection des renseignements personnels
Santé Canada et Agence de la santé publique du Canada / Gouvernement du Canada
samantha.mckay@canada.ca

From: **Praine, Lisa (HC/SC)** lisa.praine@canada.ca
Subject: RE: Access to Information Consultation from Health Canada - A-2018-001795
Date: December 8, 2020 at 8:48 AM
To: Ghassan Saed gsaed@med.wayne.edu

LP

Good morning,

I just wanted to follow up on the consultation that we sent to you. Please let me know when we can expect your response (by email is fine).

Feel free to call or email me if you want to discuss.

Thank you,

Lisa Praine

Manager, Access to Information and Privacy
Health Canada and Public Health Agency of Canada / Government of Canada
lisa.praine@canada.ca / Tel: 343-542-5320 / Fax: 613-941-4541

Gestionnaire, Accès à l'information et protection des renseignements personnels
Santé Canada et Agence de la santé publique du Canada / Gouvernement du Canada
lisa.praine@canada.ca / Tél : 343-542-5320 / Télécopieur 613-941-4541

From: Ghassan Saed <gsaed@med.wayne.edu>
Sent: 2020-10-22 1:46 PM
To: Praine, Lisa (HC/SC) <lisa.praine@canada.ca>
Subject: Re: Access to Information Consultation from Health Canada - A-2018-001795

Hi Lisa,
I have created an account for Epost that is linked to email gsaed87@yahoo.com.
Best regards
Ghassan Saed

On Oct 13, 2020, at 1:39 PM, Praine, Lisa (HC/SC) <lisa.praine@canada.ca> wrote:

[EXTERNAL]

Good afternoon,

I work in the Access to Information and Privacy Division of Health Canada. I am currently working on an Access to Information request and I have come across some documents that originated with your organization. I am preparing a consultation to send to your for your recommendations as to their release to our applicant.

Given our current work conditions (working from home), I am wondering if you would be willing to sign up for EPOST, at no cost to you, so that I could send the records to you securely. Sending information via EPOST is

much more secure than sending it by regular email. Please let me know if/when you are willing to set up and EPOST account and to which email address I can send the records in EPOST. Please find attached a document with more information about EPOST.

If you would prefer to receive the documents by courier, please provide me with your complete mailing address.

I await your response.

Please feel free to call or email me if you have any questions.

Thank you,

Lisa Praine

Manager, Access to Information and Privacy
Health Canada and Public Health Agency of Canada / Government of Canada
lisa.praine@canada.ca / Tel: 343-542-5320 / Fax: 613-941-4541

Gestionnaire, Accès à l'information et protection des renseignements personnels
Santé Canada et Agence de la santé publique du Canada / Gouvernement du Canada
lisa.praine@canada.ca / Tél : 343-542-5320 / Télécopieur 613-941-4541

<epost poster_HC-SC.docx>

Dr. Ghassan M. Saed
Associate Professor of Gynecologic Oncology
Director of Ovarian Cancer Biology Research
Departments of Obstetrics and Gynecology and Oncology
Member of Tumor Biology and Microenvironment Program
Karmanos Cancer Institute
Wayne State University School of Medicine
Detroit, MI 48202
(313) 577-5433 Office
(313) 577-1302 Lab
(313) 577-4633 Fax

Date of Preparation: September 16, 2021

Signature

GHASSAN M. SAED, Ph.D.

Associate Professor with Tenure (Research)

OFFICE ADDRESS: The C.S. Mott Center for Human Growth and Development
Department of Obstetrics and Gynecology
275 East Hancock Avenue
Detroit, MI 48201

OFFICE TELEPHONE NUMBER: (313) 577-5433

OFFICE FAX NUMBER: (313) 577-8554

EMAIL ADDRESS: g.saed@wayne.edu

EDUCATION:

Ph.D. in Molecular Biology University of Essex, Colchester, England, United Kingdom	1983–1986
B.S. in Biochemistry King Saud University, Riyadh, Saudi Arabia	1979–1982

POSTGRADUATE TRAINING:

Fellowship in Immunopathology, University of Michigan, Ann Arbor, MI	1992–1993
Fellowship in Molecular Biology, Henry Ford Hospital, Detroit, MI	1988–1990

FACULTY APPOINTMENTS:

Adjunct Associate Professor, Department of Oncology, Karmanos Cancer Institute, Detroit Medical Center/Wayne State University School of Medicine, Detroit, MI	2017–Present
Director, Ovarian Cancer Biology Research, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI	2009–Present
Scientific Member, Karmanos Cancer Institute, Molecular Biology and Genetics Program, Wayne State University School of Medicine, Detroit, MI	2008–Present
Member of Tumor Biology and Microenvironment Program, Karmanos Cancer Institute, Detroit, MI	2008–Present
Associate Professor (secondary), Department of Physiology, Wayne State University School of Medicine, Detroit, MI	2008–Present

Associate Professor (primary), Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI	2007–Present
Tenure, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI	2007–Present
Associate Status, Department of Anatomy/Cell Biology, Wayne State University School of Medicine, Detroit, MI	2003–Present
Tenure Track, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI	2001–2007
Assistant Professor, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI	1998–2007

HOSPITAL OR OTHER PROFESSIONAL:

Senior Investigator, Center for Biomedical Research, Oakland University, Rochester, MI	1997–1998
Adjunct Associate Professor, Department of Chemistry, Oakland University, Rochester, MI	1996–2004
Bioscientific Staff Investigator, Dermatology Department, Henry Ford Hospital, Detroit, MI	1995–1998
Associate Staff Investigator, Department of Dermatology, Henry Ford Hospital, Detroit, MI	1993–1994
Special Lecturer, Department of Chemistry, Oakland University, Rochester, MI	1991–1996
Assistant Staff Investigator, Hypertension Research, Henry Ford Hospital, Detroit, MI	1990–1992

MAJOR PROFESSIONAL SOCIETIES

Member, Society for Gynecologic Oncology	2017–Present
Member, American Association of Cancer Research	2008–Present
Member, American Federation of Clinical Research	2009–Present
Member, American Society for Reproductive Medicine	1998–Present
Member, Society for Reproductive Investigation	1998–Present
Member, American Association of University Professors	1996–Present
Member, American Chemical Society	1991–2014
National Research Council of the United Kingdom	1985–1986
Medical Research Council of the United Kingdom	1984–1998

HONORS/AWARDS:

Oncology Research fellowship — 2017

Emi Bulica, awarded a year-off oncology research scholarship from Michigan State University in the laboratory of Dr. Saed of the Department of Obstetrics & Gynecology at Wayne State University. This award is for 12 months of full-time research in Dr. Saed's laboratory studying ovarian cancer.

Star Award — 2017

73rd American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo

This award recognizes members who have presented during at least nine of the ASRM Annual Meetings from the years 2007-2016. Presentations

may include Congress courses and/or seminars, Scientific Program symposia, posters, and/or oral presentation of abstracts. 234 awardees

SRS In-Training Award for Research 2017
Awarded to Nicole King, PhD, Postdoctoral Fellow in the laboratory of Dr. Ghassan Saed
73rd American Society for Reproductive Medicine (ASRM Scientific Congress & Expo
The purpose of this award is to recognize outstanding research conducted by individuals in training under the "Reproductive Surgery" category. He/she is a presenting first author, and a medical student, resident, fellow, or under-graduate, graduate, or postdoctoral student. Three awardees

Star Award 2016
72nd American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo
This award recognizes members who have presented during at least nine of the ASRM Annual Meetings from the years 2007-2015. Presentations may include Congress courses and or seminars, Scientific Program symposia, posters, and/or oral presentation of abstracts. ~235 awardees

Excellence in Biomedical Research 2015
Global Medical Discovery Series
Key Scientific Article for peer-reviewed publication entitled: "Sox2 Gene Amplification Significantly Impacts Overall Survival in Serous Epithelial Ovarian Cancer." Reproductive Sciences 22(1):38-46, 2015. Epub July 18, 2014. One awardee

Star Award 2013
69th American Society for Reproductive Medicine Annual Meeting (ASRM)
This award recognizes members who have presented during at least nine of the ASRM Annual Meetings from the years 2007-2012. Presentations may include Congress courses and/or seminars, Scientific Program symposia, posters, and/or oral presentation of abstracts. ~235 awardees

Star Award 2011
67th American Society for Reproductive Medicine (ASRM) Annual Meeting
This award recognizes members who have presented during at least nine Of the ASRM Annual Meetings from the years 2007-2010. Presentations may include Congress courses and/or seminars, Scientific Program symposia, posters, and/or oral presentation of abstracts. ~235 awardees

Award for Graduate Students Who Obtained External Support 2010
Jennell White, Obstetrics/Gynecology
Advisor: Ghassan Saed, Obstetrics/Gynecology
Agency: National Institutes of Health

President's Award for Excellence in Teaching 2009
Wayne State University School of Medicine

This award is in recognition for outstanding faculty who have made contributions to teaching at WSU to an exceptionally high degree, demonstrate comprehensive knowledge of their subject, superior classroom performance, and high educational standards; communicate their subject matter accurately, clearly, and effectively; generate enthusiasm and respect for learning; motivate their students to excel; and are accessible to students; innovative instructional practices, impact on teaching at WSU, and contributions to advancing teaching in their field.

Finalist Paper 2006
62nd American Society for Reproductive Medicine (ASRM) Annual Meeting
One awardee

Travel Award winner 2006
1st International Conference on Ovarian Cancer: State of the Art and Future Directions, Aegean conferences, Greece

Prize Paper Candidate 2005
Conjoint 61st American Society for Reproductive Medicine (ASRM) Annual Meeting and 51st Canadian Fertility and Andrology Society Annual Meeting
One awardee

Finalist Paper 2005
61st American Society for Reproductive Medicine (ASRM) Annual Meeting
One awardee

Finalist Paper 2003
Society of Reproductive Endocrinology and Infertility (SREI) Annual Meeting
One awardee

Finalist Paper, Basic Science 2003
19th European Society of Human Reproduction and Embryology (ESHRE) Annual Meeting
One awardee

Award Paper 2000
58th Society of Reproductive Surgeons (SRS) Scientific Program

Finalist Paper 2000
Society of Reproductive Endocrinology and Infertility (SREI) Annual Meeting
One awardee

Award Paper 1998
52nd Society of Reproductive Surgeons (SRS) Scientific Program

Outstanding Professor of the Year Award 1996–1997
Golden Key National Society, Oakland University Chapter, Rochester, MI
One awardee

SERVICE:

Wayne State University

Departmental/Divisional

Chairperson, Organizing Committee, 2017 Joint Annual Reproductive Sciences Retreat, Departments of Obstetrics and Gynecology, Wayne State University School of Medicine and University of Toronto; and The Michigan Alliance for Reproductive Technologies and Sciences (MARTS) Annual Meeting at Wayne State University	2017
Faculty Mentor, NIH/NICHD Women's Reproductive Health Research (WRHR) Scholar Program, Department of Obstetrics and Gynecology	2012–2016
Faculty Associate, Fulbright Visiting Senior Scholar Award recipient Dr. Iyad Ali, Department of Obstetrics and Gynecology	2012–2014
Member, Selective Salary Committee, Department of Obstetrics and Gynecology	2012–Present
Member, Promotion and Tenure Committee, Department of Obstetrics and Gynecology	2012–Present
Chairperson, C.S. Mott Center Seminar Series Committee, Department of Obstetrics and Gynecology	1998–Present
Chairperson, Basic Research Endocrine Fellows Training Committee, Department of Obstetrics and Gynecology	1998–2014
Member, Reproductive Endocrinology and Infertility Fellowship Selection Committee, Department of Obstetrics and Gynecology	1998–Present

School of Medicine

Chair, Master committee for Osama Nusrat, MD, MS candidate, Department of Physiology in the Reproductive Sciences Wayne State University School of Medicine.	2015-2017
Member, Strategic Research Initiative Grant Review (SRIG) Committee, Karmanos Cancer Institute	2013–2014
Member, PhD Committee for Batoul Abdullah, PhD Candidate, Center for Molecular Medicine and Genetics, Wayne State University	2012–2016
Chair, PhD Committee for Jimmy Belotte, MD, PhD Candidate, Department of Physiology and Reproductive Sciences, Wayne State University School of Medicine.	2012–2016
Faculty, Reproductive Sciences Graduate Program, Department of Physiology, Wayne State University	2012–2016

Chair, PhD committee for Nicole M. Fletcher, PhD candidate Department of Physiology and Reproductive Sciences, Wayne State University School of Medicine.	2008-2013
Chair, PhD committee for Jennell White Jackson, PhD candidate, Department of Physiology in the Reproductive Sciences, Wayne State University School of Medicine.	2000-2011
Member, Search Committee for a candidate selection for a joint appointment in the Departments of Psychology and Obstetrics & Gynecology in the field of Psychopharmacology	2003–2005

Affiliate Medical Organizations

National Arab American Medical Association: Serve as Chief Financial Officer, Board Member, and two times President of Michigan Chapter.	2009–Present

Professional

Member for publication committee at the Society of Reproductive Investigation	2019-Present
Society for Gynecologic Oncology: Serve as judge for oral and poster presentations and abstract selection committee.	2017-Present
Karmanos Cancer Institute: Design research projects and give lectures in the Molecular Biology and Genetics Program.	2008–Present
Karmanos Cancer Institute: Design, discuss and present research projects for the Tumor Biology and Microenvironment Program.	2007–Present
Society for Reproductive Investigation: Serve as judge for oral and poster presentations and abstract review committee.	2005-Present
American Society for Reproductive Medicine: Serve as judge for oral and poster presentations, and abstract review committee.	2006-Present
Yale University, New Haven, CT: Served as a member in Scientific Review Committee, Ethel F. Donoghue Women's Health Investigator Program,	2004-2004
Henry Ford Hospital, Detroit, MI: Served in Biohazard and Safety Committee, Radioisotope Safety Committee, Animal Care Committee, Residents/Fellows Basic research training and education committee	1993-1998

Consulting

Consultant – Collaborator for the Department of Obstetrics and Gynecology, University of Illinois, Chicago, IL	2017–Present
Consultant and Witness Expert on behalf of plaintiffs in national litigation	2017–Present

against Johnson & Johnson Baby Powder and increased risk of ovarian cancer

Consultant – Collaborator for the Department of Obstetrics and Gynecology, University of Tennessee Health Science Center, Memphis, TN 2012–Present

Consultant – Collaborator for the Department of Obstetrics and Gynecology, University of Augusta, GA 2013–Present

Consultant, Molecular Biologic Testing, DS Biotech, Detroit, MI 2013–Present

Consultant, Application of Cyclooxgenase-2 in the Treatment of Ovarian Cancer, Pfizer Pharmaceuticals, Rochester, MI 2002-2003

Consultant, Technical expertise in developing molecular probes and Markers, Oxford Biomedical Research, Oxford, MI 1991–1998

Scholarly Service

Grant Review Committees

Member, Scientific Review Committee, Ethel F. Donoghue Women's Health Investigator Program, Yale University, New Haven, CT 2004

Service for Peer-Reviewed Journals Editorship

Editorial Board Membership:

Editor-in-Chief, Gynecology and Obstetrics Research-Open Journal 2015–Present

Review of Manuscripts and Chapters:

Journal of Cellular and Molecular Medicine	2015–Present
Systems Biology in Reproductive Medicine	2013–Present
Journal of Assisted Reproduction and Genetics	2013–Present
Journal of Reproductive Science	2012–Present
European Journal of Obstetrics & Gynecology and Reproductive Biology	2009
Gastroenterology	2007
Houghton Mifflin Company, College Division	2003
American Gynecological and Obstetrical Society	2003
Oncogenes	2003
Fertility and Sterility	2001–Present
Wound Repair and Regeneration	2000–Present
Journal of Cytokine Research	1998–2000

TEACHING

Teaching at Wayne State University

Undergraduate Students

Instructor. Department of Biological Sciences – BIO 3990: Undergraduate course primarily for biology majors who wish to continue in a field beyond that covered in regular courses under the direction of Biological Sciences faculty.

Instructor and Advisor. Department of Physiology – PSL 5010: Undergraduate course involving student participation in laboratory research in the physiological sciences under the supervision of a departmental faculty advisor.
This course involves an introduction to experimental protocol and current related scientific literature.

Advisor. Department of Biological Sciences – BIO 6990: Undergraduate course for honors students involving student participation in laboratory research in the physiological sciences under the supervision of a departmental faculty advisor.

Graduate Students

Instructor. Interdisciplinary Biomedical Sciences – IBS 7060: Biomedical Endocrine and Reproductive Systems Development.
This course is for graduate students within the Ph.D. Program in Anatomy and Cell Biology of which has the aim of providing a broad-based knowledge of the important areas of biomedical research.

Instructor. Department of Physiology with Concentration in the Reproductive Sciences Program (PhD) – RPS 7350: Biomolecular Techniques: From Genes to Protein

Instructor. Department of Physiology with Concentration in the Reproductive Sciences Program (PhD), Principles of Reproductive Biology – PSL 7690: Cancers in Reproductive Organs/ Journal Club.
This lecture explains the impact of cancer in women; to discuss the epidemiology, risk factors, screening modalities and preventative strategies of gynecologic cancers and the role of stem cells.

Instructor. Current Research Topics in the Reproductive Sciences – PSL 7775: Molecular Mechanisms of Postoperative Adhesions.
This course is for graduate students within the Ph.D. Program in Physiology with Concentration in the Reproductive Sciences of which covers current research topics in reproductive sciences. The Program itself incorporates the teaching, research and physical resources of both the Physiology and the Obstetrics and Gynecology Departments, offering interdisciplinary doctoral training in a clinical environment in the reproductive sciences. The primary academic focus engages teaching and research training in reproduction and development, with an emphasis on the following: developmental biology, perinatal biology, reproductive endocrinology, reproductive genetics, toxicology/teratology and molecular biology including genomics, proteomics, and bioinformatics. Dissertation research is under the mentorship of Obstetrics and Gynecology basic science graduate faculty.

Advisor and Mentor. Current Research Topics in the Reproductive Sciences – PSL 7996: Arranged Research.
This course is for the graduate students within the “Ph.D. Program in Physiology with Concentration in the Reproductive Sciences” (as described in PSL 7775) which covers graduate level experiences in research techniques. It is required that special research topics, within specified areas, be agreed upon between individual faculty members and students.

Advisor and Mentor. Doctoral Candidate Status I-IV – PSL 9991, 9992, 9993, 9994: Thesis/Dissertation Research and Design.
This course is for the graduate students within the "Ph.D. Program in Physiology with Concentration in the Reproductive Sciences" (as described in PSL 7775). Required in consecutive academic-year semesters following advancement to Ph.D. candidacy status I through IV.

Advisor and Mentor. Doctoral Candidate Dissertation Research and Direction – PSL 9995: Candidate Maintenance Status.
This course is for the graduate students within the "Ph.D. Program in Physiology with Concentration in the Reproductive Sciences" as described above in PSL 7775. Required after completion of 30 credits in PSL 9991-9994.

Director. Summer Reproductive Technology Course. The CS Mott Center for Human Growth and Development, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, 2014-2015.
The course design is to allow for Reproductive Endocrinology and Infertility/Medical Genetics fellows, as well as graduate students to become familiar with laboratory techniques in the reproductive sciences. The graduate students will acquire a thorough understanding of the theory and special methodology utilized to perform techniques indicative of reproductive endocrinology and infertility.

Lecturer. Laboratory Techniques. Summer Reproductive Technology Course. The CS Mott Center for Human Growth and Development, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, 2014.
This lecture explains the various laboratory techniques, and their limitations, as applied to the reproductive sciences.

Lecturer. Molecular Biological Procedures. Summer Reproductive Technology Course. The CS Mott Center for Human Growth and Development, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, 2015.
This lecture explains the various laboratory techniques, and their limitations, as applied to the reproductive sciences.

Residents and Fellows

Director. Summer Reproductive Technology Course. The CS Mott Center for Human Growth and Development, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, 2014-2015.
The course design is to allow for Reproductive Endocrinology and Infertility/Medical Genetics fellows, as well as graduate students to become familiar with laboratory techniques in the reproductive sciences. The fellows will acquire a thorough understanding of the theory and special methodology utilized to perform techniques indicative of reproductive endocrinology and infertility.

Instructor. PCR Technique: Concept and Clinical Application Course. The CS Mott Center for Human Growth and Development, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, 2012-Present.
This course is designed to allow departmental residents, fellows, Reproductive Endocrinology and Infertility/Medical Genetics fellows, and interested graduate students (within the C.S. Mott Center) to become familiar with the PCR technique and how to use it effectively within the laboratory.

Teaching at Other Institutions

Undergraduate Students

Adjunct Associate Professor. Taught two undergraduate courses, CHM104 "Introduction to Chemical Principles" and CHM201 "Introduction to Organic and Biological Chemistry" for nursing and health sciences students at the Department of Chemistry, Oakland University, Rochester, MI, 1991-2004.

Graduate Students

Instructor. Four-day workshop: PCR Techniques, Concepts and Applications. Howard Hughes Research Program, Oakland University, Rochester, MI, May 19-22, 1998.
This workshop was for graduate, postdoctoral, laboratory research personnel, and faculty within the field of science and research.

Instructor. Taught a graduate course CHM554 "Molecular Biology and Biotechnology" at the Department of Chemistry, Oakland University, Rochester, MI, 1995-1998.

Instructor. Biotechnology: From Genes to Proteins. Department of Dermatology, Oakland University, Rochester, MI, 1993-1998.
This course was part of the Research Training in Biotechnology Program postgraduate curriculum for residents and fellows to utilize state-of-the-art molecular technology techniques to answer questions related to molecular pathogenesis of skin diseases such as skin cancer, fibrosis and wound healing.

Teaching Assistant. Introduction to Chemical Principals. Department of Chemistry, University of Essex, Colchester, England, United Kingdom, 1987-1988.

Residents and Fellows

Instructor and Laboratory Advisor. Biotechnology Research Training. Department of Dermatology, Oakland University, Rochester, MI, 1993-1998.
This program trained dermatology residents to utilize state-of-the-art molecular technology techniques to answer questions related to molecular pathogenesis of skin diseases such as skin cancer, fibrosis and wound healing.

Mentorship

Mentor on research projects related to endometriosis, postoperative adhesions, and ovarian cancer to the Department of Obstetrics and Gynecology past and present undergraduate and graduate students, residents, clinical and postgraduate fellows, scholars, faculty, and research technicians, assistants and associates.

Undergraduate Students:

Yousif Abbiss, Newaj Abdullah, Dana Abufarha, Shadi Abuolba Ahmad [awarded the 2007 Wayne State University School of Medicine Undergraduate Research Scholarship Award], Dabaja Ahmed, Ali Alarab, Radi Al-Dasouqi, Danna Al-Hadidi, Jeremy Berman, Chelsea Fortin, Ellory Greenberg, Waseem Imann, Shucni Jain, Marisa Karcz, Hadil Katato, Reem Khazaal, Yanamandra Krishnakant, Ira Memaj, Wasfeh Musheinish, Bailey Neubauer, Osama Nusrat, Tessy Oommen,

Norman Orabi, Alex Papadellis, Sonica Rehan, Mohammad Saed, James Waleke [WSU School of Medicine 2004 graduate], Rani Yaldo, Yousif Younan, Nabaa Zalzala, and Xuping (Sherry) Zhu.

Graduate:

Batoul Abdullah, PhD: ***Abdallah BY**, *Horne SD, *Stevens JB, *Liu G, *Ying AY, *Vanderhyden B, Krawetz SK, Gorelick R, Heng HH (2013). Single cell heterogeneity: Why unstable genomes are incompatible with average profiles. Cell Cycle 12:3640-3649, 2013. PMID: 24091732 PMCID: PMC3903715
Role: Mentor/Advisor

Jimmy Belotte, MD, PhD: ***Belotte J**, *Fletcher NM, *Saed MG, *Abusamaan MS, *Dyson G, Diamond MP, **Saed GM**. A single nucleotide polymorphism in catalase is strongly associated with ovarian cancer survival. PLoS One 10(8):e0135739, 2015. eCollection 2015. PMID: 26301412 PMCID: PMC4547699

Amy Harper, MD: *Fletcher NM, ***Harper A**, *Memaj I, *Fan R, Morris RT, **Saed GM**. Molecular basis supporting the association of talcum powder use with increased risk of ovarian cancer. Reproductive Sciences. In press, 2019.

Nicole King, PhD: ***Fletcher NM**, *Belotte J, *Saed MG, *Memaj I, Diamond MP, Morris RT, **Saed GM**. Specific point mutations in key redox enzymes are associated with chemoresistance in epithelial ovarian cancer. Free Radical Biology and Medicine 102:122-132, 2017. PMID: 27890641

Osama Nusrat, MD, MS: ***Nusrat O**, *Belotte J, *Fletcher NM, *Memaj I, *Saed MG, Diamond MP, **Saed GM**. The role of angiogenesis in the persistence of chemoresistance in epithelial ovarian cancer. Reproductive Sciences 23(11):1484-1492, 2016. PMID: 27122375

Jennell White, PhD: ***White JC**, *Jiang ZL, Diamond MP, **Saed GM**. Macrophages induce the adhesion phenotype in normal peritoneal fibroblasts. Fertility and Sterility 96(3):758-763.e3, 2011. Epub July 27, 2011. PMID: 21794857

Residents

Drs. (MD) Zeynep Alpay, Dana Ambler, Tarek Dbouk, Eslam Elhammady, and Valerie Shavell.

Clinical Faculty and Clinical Postgraduate Fellows:

Drs. (MD) – Faculty: Awoniyi Awonuga, Associate Professor; Jimmy Belotte, Associate Professor; and Christopher Bryant, Associate Professor; Fellows: Mazen Abdallah, Jashoma Banerjee, Alan Bolnick, Jay Bolnick, Charalampos (Harry) Chatzicharalampous, Subodhsingh Chauhan, Laura Detti, Michael Freeman, April Gago, Amy Harper, Roohi Jeelani, Sana Khan, Mohamed Mitwally, Valerie Shavell, Mili Thakur, Rahi Victory, and Terri Woodard.

Of note, the aforementioned that have participated in premier scientific meetings such as: The American Society for Reproductive Medicine; American Gynecologic and Obstetrical Society; Society for Free Radical Biology and Medicine; Society for Reproductive Investigation; Society for Gynecologic Oncology; Pacific Coast Reproductive Society; Society for the Study of Reproduction; American Association for Cancer Research; Central American College of Obstetrics and Gynecology; and American College of Obstetrics and Gynecology, as well as publishing their many

scientific achievements (articles and abstracts) in the aforementioned preeminent, peer-reviewed journals (see Publications section, pg. 27). They have also participated and presented at local events: DMC/WSU Graduate Research Day; WSU Department of Obstetrics and Gynecology Resident and Fellow Day; WSU Obstetrics and Gynecology, CS Mott Center Reproductive Sciences Retreat; and the Michigan Alliance for Reproductive Technologies and Sciences (MARTS) Symposium.

Acknowledgements:

Roohi Jeelani, MD [fellowship 2015-2017] and Sana Khan, MD [fellowship 2013-2016] of the Reproductive Endocrinology and Infertility Fellowship Program jointly received the Second Place Award for Outstanding Paper Presentation at the 65th Annual Meeting of the Pacific Coast Reproductive Society, Indian Wells, CA, in March of 2017. Their oral presentation was entitled, *"Cyclophosphamide and Its Metabolite Impact on Fertilization through Mitochondrial Dysfunction."*

Mili Thakur, MD [fellowship, 2014-2017] of the combined Reproductive Endocrinology and Infertility and Medical Genetics Fellowship Program received the First Place Award at the Department of Obstetrics and Gynecology Resident and Research Fellow Day, Wayne State University School of Medicine, Detroit, MI, in April of 2017. This award to Dr. Thakur was for her oral presentation entitled, *"Galactose and Its Metabolites Deteriorate Metaphase II Mouse Oocyte Quality and Subsequent Embryo Development by Disturbing the Spindle Structure."*

Mili Thakur, MD [fellowship, 2014-2017] of the combined Reproductive Endocrinology and Infertility and Medical Genetics Fellowship Program (the only one of its kind in the country), was the recipient of the 2016 Pfizer-SRI (Society for Reproductive Investigation), President's Presenter's Award. This award for given to Mili for her abstract entitled, "*Galactose and Its Metabolites Deteriorate Metaphase II Mouse Oocyte Quality through a Mechanism that Involves the Generation of Reactive Oxidative Species, Mitochondrial Dysfunction and Apoptosis*." The President's Presenter's Award is given in recognition of the 25 most meritorious abstracts (either poster or oral presentation) submitted by individuals still in training. Dr. Thakur received this prestigious award at the 63rd Annual Meeting of the Society for Reproductive Investigation, Montreal, Quebec, Canada, in March of 2016.

Alan Bolnick, MD and Sana Khan, MD [fellowship, 2013-2016] of the Reproductive Endocrinology and Infertility Fellowship Program were each awarded, from the Pacific Coast Reproductive Society, the 2015 Travel Award; as well as Roohi Jeelani, MD and Mili Thakur, MD [fellowship, 2015-2017 and 2014-2017, respectively] who were each awarded the 2016 Travel Award. These travel awards paid for registration to the annual meeting, course fees, and all travel expenses incurred.

Michael Freeman, MD [fellowship, 1999-2002] of the Reproductive Endocrinology and Infertility Fellowship Program was the recipient of a $20,000 research grant from the American Gynecologic and Obstetrical Society (AGOS).

Scholars

Iyad Ali, PhD: Assistant Professor of Biochemistry, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine. Visiting Fulbright Arab Fund Fellowship Scholar [2013-2014] in the laboratories of Drs. Husam Abu-Soud and Ghassan Saed, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Wayne State University School of Medicine. Citation: *Maitra D,* **Ali I**, *Abdulridha RM, *Shaeib F, *Khan

SN, **Saed GM**, Pennathur S, Abu-Soud HM. PLoS One 9(11):e110595, 2014. eCollection 2014. PMID: 25375773

Awoniyi Awonuga, MD: Associate Professor, Women's Reproductive Health Research (WRHR) Scholar [2012-2015], Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Wayne State University School of Medicine. Citation: ***Awonuga AO**, *Belotte J, *Abuanzeh S, *Fletcher NM, Diamond MP, **Saed GM**. Advances in the pathogenesis of adhesion development: the role of oxidative stress. Reproductive Sciences 21(7):823-836, 2014. Epub February 11, 2014. Review. PMID: 24520085 PMCID: PMC4107571

Jimmy Belotte, MD, PhD: Associate Professor, Women's Reproductive Health Research (WRHR) Scholar [2012-2016], Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Wayne State University School of Medicine. Citation: ***Belotte J**, *Fletcher NM, *Alexis M, Morris RT, Munkarah AR, Diamond MP, **Saed GM**. Sox2 gene amplification significantly influences overall survival in serous epithelial ovarian cancer. Reproductive Sciences 22(1):38-46, 2015. Epub July 18, 2014. PMID: 25038052 PMCID: PMC4275450

Lylia Fahmy, MD: Clinical Instructor, Women's Reproductive Health Research (WRHR) Scholar [2001-2003], Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Wayne State University School of Medicine. Thesis: Effect of Ovarian Hormones on Adhesion Development.

Faculty

Mentor of current and past Obstetrics and Gynecology clinical faculty through collaborations on research projects and grant submissions. Faculty members are as follows: Awoniyi Awonuga, MD, Professor, Division of Reproductive Endocrinology and Infertility; Jimmy Belotte, MD, PhD, Associate Professor [past], Division of Gynecology; Christopher Bryant, MD, Associate Professor [past], Division of Gynecology; Lylia Fahmy, MD, Clinical Instructor [past], Division of Reproductive Endocrinology and Infertility; and Peter Baumann, MD, Associate Professor [retired], Division of Gynecology.

I have also been instrumental to key professional presentations at local, national, and international conferences by our past and present senior faculty members of the Obstetrics and Gynecology Department. They are as follows: Jay Berman, MD, Associate Professor and Associate Chair, Director, Division of Gynecology; Michael Diamond, MD, Professor and Associate Chair [past], Director, Division of Reproductive Endocrinology and Infertility [past], and Assistant Dean of Clinical and Translational Research [past, now at Georgia Regents University]; Bernard Gonik, MD, Professor, Division of Maternal and Fetal Medicine; John Malone, Jr, MD, Professor and Chair [past, deceased]; Kamran Moghissi, MD, Professor Emeritus and Chair Emeritus [past], Director, Division of Reproductive Endocrinology and Infertility [past], and Director, CS Mott Center for Human Growth and Development [past, retired]; and Adnan Munkarah, MD, Professor and Director, Division of Gynecologic Oncology.

Research Associates/Assistants/Technicians

In the laboratory of Dr. Ghassan Saed: Drs. (PhD) Boytcho Boytchev, Nicole King, Semira Galijasevic, Zhongliang (John) Jiang, MD, Hong Lu, Qui Lu, Gheorghe Proteasa, Natalie Rizk, Rona Wang, MD, and Ming Zhao, MD; Danielle Hall, BS, Ira Memaj, BS, and Manal Omar, BS.

Essays/Theses/Dissertations Directed

Osama Nusrat, MD, MS, Department of Physiology in the Reproductive Sciences (2015-2017), Wayne State University School of Medicine, Detroit, MI
Dissertation Title: The Role of Angiogenesis in the Persistence of Chemoresistance in Epithelial Ovarian Cancer
Date Awarded: Master degree, September 2017
Current Status: Resident, Department of Internal Medicine, University of Arizona College of Medicine, Tucson, AZ

Jimmy Belotte, MD, PhD, Associate Professor, Department of Physiology in the Reproductive Sciences Concentration (2012-2016); and Women's Reproductive Health Research (WRHR) Scholar, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI
Dissertation Title: The Role of Oxidative Stress in the Establishment of Resistance to Cisplatin in Epithelial Ovarian Cancer Cells
Date Awarded: PhD Degree, Sept. 14, 2016 and WRHR training completed Sept. 2016
Current Status: Associate Professor, Department of Obstetrics and Gynecology, Division of Gynecology, Montefiore Medical Center, Bronx, NY

Batoul Abdullah, PhD, Department of Physiology in the Center for Molecular Medicine and Genetics Concentration (2012-2016), Wayne State University School of Medicine, Detroit, MI
Dissertation Title: Fuzzy Inheritance: A Novel Form of Somatic Cell Inheritance that Regulates Cell Population Heterogeneity.
Date Awarded: PhD Degree, 2016
Current Status: Postdoctoral Fellow in the laboratory of Henry (Hong-Qiang) Heng, PhD, Center for Molecular Medicine & Genetics and Pathology, Wayne State University School of Medicine, Detroit, MI

Awoniyi Awonuga, MD, Women's Reproductive Health Research (WRHR) Scholar [2012 2015], Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI
Thesis Title: Oxidative Stress in the Pathogenesis of Post-Operative Adhesions
Training Completed: December 2015
Current Status: Professor and Interim Director, Division of Reproductive Endocrinology and Infertility and Director, Residency Program Research, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI

Nicole King, PhD, Department of Physiology in the Reproductive Sciences Concentration Program (2008-2013), Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI
Dissertation Title: The Role of Oxidative Stress in the Pathogenesis of Epithelial Ovarian Cancer
Date Awarded: PhD Degree, August 2013
Current Status: Postdoctoral Fellow in the laboratory of Ghassan M Saed, PhD, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, CS Mott Center for Human Growth and Development, Wayne State University School of Medicine, Detroit, MI

Jennell White Jackson, PhD, Department of Physiology in the Reproductive Sciences Concentration Program (2000-2011), Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI
Dissertation Title: The Potential Role of Innate Immunity in the Pathogenesis of Postoperative Adhesions
Date Awarded: PhD Degree, September 2011
Current Status: Postdoctoral Fellow, Department of Pediatrics, Wayne State University School of Medicine, Detroit, MI

Lylia Fahmy, MD, Women's Reproductive Health Research (WRHR) Scholar [2001-2003], Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI
Thesis Title: Effect of Ovarian Hormones on Adhesion Development
Training Completed: September 2003
Current Status: Associate Professor, Department of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, NB

Course or Curriculum Development

Originator and Director. Summer Reproductive Technology Course. 2014
This course design is to allow for Reproductive Endocrinology and Infertility/Medical Genetics fellows, as well as graduate students, to become familiar with all aspects of laboratory techniques within the field of reproductive sciences. Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, The C.S. Mott Center for Human Growth and Development, Wayne State University School of Medicine, Detroit, MI.

Course Director. Reproductive Sciences Concentration – RPS 7350: 2006
Biomolecular Techniques: From Genes to Protein.
This course design is specifically for graduate students enrolled in The PhD Program in Physiology with Concentration in the Reproductive Sciences, as part of their curriculum. This is an integrated PhD program incorporating the teaching, research, and physical resources of two departments -- Physiology and Obstetrics & Gynecology at Wayne State University School of Medicine, Detroit, MI.

Organizer. Four-day workshop (May 19-22): PCR Techniques, Concepts, 1998
and Applications.
Workshop developed for undergraduates, graduates, postdoctoral, laboratory personnel, and faculty studying and/or working within the field of science and research. Sponsored by the Howard Hughes Research Program of Oakland University, Rochester, MI.

Designer. Introduction to Molecular Cloning. 1996
Course designed to teach techniques for characterization and manipulation of DNA and RNA from the basis of modern biomedical research. Coursework pertinent towards medical residents and fellows at the Henry Ford Hospital, Detroit, MI, and graduate students at Oakland University, Rochester, MI.

Designer. Research Training in Biotechnology. 1993

This program trained Department of Dermatology residents and fellows to utilize state-of-the-art molecular technology techniques to answer questions related to molecular pathogenesis of skin diseases such as skin cancer, fibrosis and wound healing at Henry Ford Hospital, Detroit. MI. This training ended in 1998.

Course Director. I have participated in developing the course, Introduction to Chemical Principles (CHM 104) to meet general education requirements. CHM 104 satisfies the university general education requirement in natural science and technology (NST). The learning outcomes for NST courses state that the student will demonstrate knowledge of major concepts from natural science or technology, including developing and testing of hypotheses, drawing conclusions, and reporting of findings through some laboratory experience or an effective substitute. This course taught at Oakland University, Rochester, MI. 1991–2004

Designer. Laboratory course. I was actively involved in developing and instructing two laboratory sections for CHM 104. Students learned how to evaluate sources of information in science or technology. Developed at Oakland University, Rochester, MI 1991–2004

Designer. I developed and taught CHM 104 and CHM 201 to nursing students on-line (a web-based instruction). I designed courses to satisfy the university general education requirement in natural science and technology (NST). For this, I utilized and implemented the virtual chemistry laboratory experience to be an integral part of this course. Developed at Oakland University, Rochester, MI, 2005–2010

GRANTS, CONTRACTS, AND OTHER FUNDING:

Active National/International Grants and Contracts
Role: Principal Investigator, Percent Effort: 10%
Title: Novel target for ovarian cancer treatment
Source: DS Biotech LLC.
Date: 01/01/2021- 12/31/2021
Total Direct Costs: $100,000

Pending National/International Grants and Contracts
None

Submitted National/International Grants and Contracts

Role: Principal Investigator, Percent Effort: 30%
Title: Monomeric MPO as a biomarker for early detection of ovarian cancer
Source: NIH/NICHD R01
Date: 09/01/2020 – 08/31/2025
Total Direct Costs: $1,250,000

Role: Principal Investigator, Percent Effort: 30%
Title: Novel mechanism of survival in EOC cells
Source: NIH/NICHD R21

Date: 09/01/2020 – 09/01/2022
Total Direct Costs: $375,000

Role: Principal Investigator, Percent Effort: 30%
Title: Binding of intracellular monomeric MPO to aV/b1 integrin serves as a novel mechanism of survival in EOC cells.
Source: NIH/NICHD R01
Date: 09/01/2019 – 08/31/2024
Total Direct Costs: $1,250,000

Role: Principal Investigator, Percent Effort: 10%
Title: Binding of intracellular monomeric MPO to aV/b1 integrin serves as a novel mechanism of survival in EOC cells.
Source: DOD
Date: 01/01/2018 – 01/01/219
Total Direct Costs: $385,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD
Title: "Novel Mechanism of Apoptosis in Chemoresistant Ovarian Cancer Cells." To determine whether chenoresistance in ovarian cancer manifests decreased apoptosis through enhanced s-nitrosylation of caspase-3 mechanism which can, thereby, be reversed by DCA.
Source: American Association for Cancer Research (AACR)
Date: 07/01/17 – 06/30/19
Total Direct Costs: $100,000

Role: Principal Investigator, Percent Effort: 30%
Title: "Identification of a Novel Target with Intriguing Anti-Tumorigenic Effects in Ovarian Cancer." To identify and test a target that cross-reacts with the CD11b antibody and determines its efficacy in killing both sensitive and chemoresistant ovarian cancer cells.
Source: NIH/NICHD R01, Proposal #17-0220
Date: 09/01/17 – 08/31/22
Total Direct Costs: $1,919,600

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD
Title: "Novel Marker of Survival in Ovarian Cancer Cells." To test the anti-tumorigenic potential of integrin αV/β1 antibodies in sensitive and chemoresistant ovarian cancer.
Source: U.S. Department of Defense (DOD)
Date: 01/01/18 – 12/31/19
Total Direct Costs: $385,000

Role: Principal Investigator, Percent Effort: 25%
Title: "Cross-Talk Between MPO and iNOS Regulates Apoptosis in Chemoresistant Ovarian Cancer." To determine whether chemoresistance in ovarian cancer manifests decreased apoptosis through enhanced s-nitrosylation of caspase-3 mechanism, which can be reversed by DCA.
Source: NIH/NICHD R21
Date: 09/01/17 – 08/31/19
Total Direct Costs: $423,500

Role: Principal Investigator, Percent Effort: 10%

Title: "A Novel Target with Intriguing Anti-Tumorigenic Effects in Cancer." To identify and test a target that cross-reacts with the CD11b antibody and determines its efficacy in killing both sensitive and chemoresistant ovarian cancer cells.
Source: NIH/DHHS Small Business Technology Transfer Grant (STTR), R41
Date: 07/01/17 – 6/30/18
Total Direct Costs: $299,999

Role: Principal Investigator, Percent Effort: NA
Title: "A Novel Target with Intriguing Anti-Tumorigenic Effects in Cancer." To identify and test a target that cross-reacts with the CD11b antibody and determines its efficacy in killing both sensitive and chemoresistant ovarian cancer cells.
Source: The Honorable Tina Brozman Foundation, Inc. for Ovarian Cancer Research – Letter of Intent
Date: 2017
Total Direct Costs: $100,000

Role: Principal Investigator, Percent Effort: 5%; Co-Principal Investigator: NM King, PhD
Title: "Potential Anti-Tumorigenic Antigen for Cancer Therapy." To identify and test a target that cross-reacts with the CD11b antibody and determines its efficacy in killing both sensitive and chemoresistant ovarian cancer cells.
Source: Elsa U. Pardee Foundation Grant Program, Proposal #17-0715
Date: 01/01/18 – 12/31/18
Total Direct Costs: $187,958

Role: Principal Investigator, Percent Effort: NA
Title: "A Novel Target with Intriguing Anti-Tumorigenic Effects in Cancer." To identify and test a target that cross-reacts with the CD11b antibody and determines it efficacy in killing both sensitive and chemoresistant ovarian cancer cells.
Source: Ovarian Cancer Research Fund Alliance, Inc. (OCRFA)
Date: 01/01/18 – 12/31/20
Total Direct Costs: $300,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD
Title: "Antitumor Effects of Targeting Integrin αV/β1 in Ovarian Cancer Cells." To test the anti-tumorigenic potential of integrin αV/β1 antibodies in ovarian cancer patient samples.
Source: Ovarian Cancer Research Fund Alliance, Inc., Ann Schreiber Mentored Investigator Award
Date: 01/01/18 – 12/31/18
Total Direct Costs: $75,000

Submitted Other Grants and Contracts

Role: Principal Investigator
Title: "Repurposing ABCIXIMAB, A Clinically Approved Anticoagulant for the Treatment of Ovarian Cancer." To determine whether abciximab is an effective therapy against sensitive and resistant ovarian cancer.
Source: Michigan Ovarian Cancer Alliance (MIOCA)
Date: 04/01/17 – 03/31/18
Total Direct Costs: $50,000

Role: Principal Investigator

Title: "ReoPro and Ovarian Cancer"
Source: Michigan Ovarian Cancer Alliance (MIOCA)
Date: 04/01/17 – 03/31/18
Total Direct Costs: $50,000

Role: Principal Investigator
Title: "A Novel Target with Intriguing Anti-Tumorigenic Effects in Cancer"
Source: DS Biotech, LLC, Proposal #17-0289
Date: 07/01/17– 06/30/18
Total Direct Costs: $100,000

Role: Principal Investigator
Title: "A Novel Target with Intriguing Anti-Tumorigenic Effects in Ovarian Cancer"
Source: Rivkin Center for Ovarian Cancer, Pilot Study Awards, 573569

Previously Funded Grants and Contracts

Role: Principal Investigator, Percent Effort: 5%
Title: "Elucidation of Cellular Mechanisms of Evitar of Post-Operative Fibrosis."
Source: Temple Therapeutics, 25S8P1
Date: 04/01/17 – 05/31/18
Total Direct Costs: $100,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD
Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The project's design is to identify key markers of oxidative stress that have the potential to serve as screening tools for ovarian cancer and may play a role in the acquisition of chemoresistance.
Source: Prevent Cancer Foundation, Postdoctoral Fellowship Grant
Date: 01/01/16 – 1/31/18
Total Direct Costs: $80,000
Role: Co-Principal Investigator; Principal Investigators: MP Diamond, MD, EN Kraiselburd, PhD
Title: "WSU-UPR Research Partnership to Promote Diversity in the Reproductive Sciences"
Source: NIH/NICHD, HD-09-008
Date: 08/2010 – 07/2015
Total Direct Costs: $3,020,000

Role: Co-Principal Investigator, Principal Investigator: MP Diamond, MD
Title: "WSU Clinical and Translational Science Award Planning Grant"
Source: NIH/NICHD, 1P20 RR 023578
Date: 09/2006 – 09/2012
Total Direct Costs: $2,225,750

Role: Consultant; Principal Investigator: MP Diamond, MD
Title: "WSU Cooperative Reproductive Medicine Network Center"
Source: NIH/NICHD, U10 HD-39005
Date: 08/2007 – 07/2012
Total Direct Costs: $1,510,000

Role: Principal Investigator, Percent Effort: 3.60%
Title: "Postoperative Adhesion: Roles of Hypoxia and Nitric Oxide"

Source: NIH/NICHD, Division of Pharmacology, Physiology, and Biological Chemistry, 1R01 GM069941-01A3
Date: 10/01/06 – 09/30/12
Total Direct Costs: $1,312,500

Role: Mentor; Principal Investigator: J White, MS, PhD Candidate (WSU)
Title: "Post-Operative Adhesions: Roles of Hypoxia in Nitric Oxide"
Source: NIH/NICHD, Minority Research Supplemental Award, 3R01GM069941-02S1
Date: 01/01/08 – 08/31/10
Total Direct Costs: $151,441

Role: *Principal Investigator
Title: "*CUAAH Subcontract – Specialty Laboratory Core"
Date: 06/01/08 – 05/31/10
Total Direct Costs: $403,840

Role: Co-Principal Investigator; Principal Investigator: JM Flack, MD
Title: "Center for Urban African American Health (CUAAH)"
Source: NIH/NIEHS
Date: 06/01/07 – 05/31/10
Total Direct Costs for Center: $9,487,709

Role: Principal Investigator
Title: "Angiogenesis of Ovarian Cancer"
Source: Frank Iacobell Endowed Chair, Department of Obstetrics and Gynecology, Wayne State University School of Medicine
Date: 01/01/08 – 12/31/09
Total Direct Costs: $41,500

Role: Co-Principal Investigator; Principal Investigator: R Kannan, PhD
Title: "Wayne State University, Department of Engineering – Subcontract"
Source: President's Research Award, Technology and Transfer Office
Date: 01/01/08 – 12/31/09
Total Direct Costs: $15,000

Role: Consultant; Principal Investigator: MP Diamond, MD U10 HD-39005
Title: "WSU Cooperative Reproductive Medicine Network Center"
Source: NIH/NICHD
Date: 04/01/00 – 03/31/07
Total Direct Costs: $1,349,994

Role: Principal Investigator; Co-Principal Investigator: MP Diamond, MD
Title: "Testing of Perfluorodecalin for Adhesion Prevention"
Source: Novel Pharma, Inc.
Date: 11/01/01 – 06/30/02
Total Direct Costs: $32,000

Role: Principal Investigator; Co-Principal Investigator: MP Diamond, MD
Title: "Effect of Tissel on Human Peritoneal Fibroblasts"
Source: Baxter Research Grant

Date: 09/30/01 – 12/31/02
Total Direct Costs: $98,000
Role: Co-Principal Investigator; Principal Investigator: MP Diamond, MD
Title: "Why Does Endometriosis Cause Adhesions?"
Source: Endometriosis Association
Date: 01/01/01 – 12/31/01
Total Direct Costs: $38,000

Role: Co-Principal Investigator; Principal Investigator: MP Diamond, MD
Title: "Effect of Tissel on Human Mesothelial Cell Culture"
Source: Baxter Research Grant
Date: 01/01/01 – 08/31/01
Total Direct Costs: $60,000

Role: Principal Investigator
Title: "The Effects of Hypoxia on the Levels of Peritoneal ECM Proteins"
Source: Wayne State University Department of Obstetrics and Gynecology, Interdepartmental Research Grant
Date: 03/01/98 – 12/31/00
Total Direct Costs: $19,000

Role: Principal Investigator
Title: "The Role of p53 in the Pathogenesis of Keloids"
Source: Henry Ford Hospital Small Project Award
Date: 01/01/98 – 12/31/98
Total Direct Costs: $20,000

Role: Principal Investigator
Title: "Patterns of Cytokine Expression in Cutaneous T-Cell Lymphoma"
Source: Henry Ford Hospital Small Project Award
Date: 01/01/93 – 12/31/94
Total Direct Costs: $20,000

Previously Submitted, Not Funded Grants and Contracts

Role: Principal Investigator, Percent Effort: 20%
Title: "Novel Mechanisms of Apoptosis in Chemoresistant Ovarian Cancer Cells." To determine whether chenoresistance in ovarian cancer manifests decreased apoptosis through enchanged s-nitrosylation of caspase-3 mechanism, which can be reversed by DCA.
Source: NIH/NICHD, R21
Date: 04/01/17 – 03/31/19
Total Direct Costs: $423,500

Role: Principal Investigator
Title: "Novel Mechanisms of Apoptosis in Chemoresistant Ovarian Cancer Cells." To determine whether chenoresistance in ovarian cancer manifests decreased apoptosis through enchanged s-nitrosylation of caspase-3 mechanism, which can be reversed by DCA.
Source: Elsa U. Pardee Foundation Grant Program
Date: 01/01/17 – 12/31/17
Total Direct Costs: $113,966

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD
Title: "Novel Mechanisms of Apoptosis in Chemoresistant Ovarian Cancer Cells." The project's design is to determine whether chemoresistance in ovarian cancer manifests decreased apoptosis through enhanced s-nitrosylation of caspase-3 mechanism, which can be reversed by DCA.
Source: NIH/NICHD, R03
Date: 12/01/16 – 11/30/18
Total Direct Costs: $50,000

Role: Principal Investigator
Title: "Innovative New Target for Ovarian Cancer Therapy." The project was designed to identify and test a target that cross-reacts with the CD11b antibody and determines its efficacy in killing both sensitive and chemoresistant ovarian cancer cells.
Source: The Honorable Tina Brozman Foundation, Inc. for Ovarian Cancer Research
Date: 08/01/16 – 07/31/18
Total Direct Costs: $200,000

Role: Principal Investigator
Title: "Redox Enzyme-Mediated Prosurvival of Chemoresistance in Ovarian Cancer." To determine whether development of chenoresistance in ovarian cancer is attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.
Source: Ovarian Cancer Research Fund Alliance, Inc. (OCRFA)
Date: 2016 – 2019
Total Direct Costs: $900,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD
Title: "Chemoresistant Ovarian Cancer Cells Manifest Lower Vascular Endothelial Growth Factor and Hypoxia Inducible Factor-1α: A Potential Survival Mechanism." The design of the project was to determine whether VEGF and HIF-1α contribute to the persistence of chemoresistance in ovarian cancer.
Source: Ovarian Cancer Research Fund, Ann Schreiber Mentored Investigator Award
Date: 2016 – 2017
Total Direct Costs: $75,000

Role: Mentor, Percent Effort: 0%; PI: NM King, PhD
Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.
Source: NIH/NICHD, R03 – Resubmission of scored proposal
Date: 12/01/15–11/30/17
Total Direct Costs: $153,583

Role: Principal Investigator, Percent Effort: 30%
Title: "Redox Enzyme-Mediated Prosurvival of Chemoresistance in Ovarian Cancers." The design of the project was to determine whether development of chemoresistance in ovarian cancer is attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.
Source: NIH/NICHD, R01
Date: 12/01/15 – 11/30/20
Total Direct Costs: $2,494,526

Role: Mentor, Percent Effort: 0%; PI: NM King, PhD

Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.
Source: NIH/NICHD, R03
Date: 11/01/14 – 10/31/16
Total Direct Costs: $152,000

Role: Principal Investigator, Percent Effort: 30%
Title: "Chemoresistance Induces a Genotype Switch in Redox Enzymes in Ovarian Cancer." The design of the project was to determine whether development of chemoresistance in ovarian cancer is attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.
Source: NIH/NICHD, R01
Date: 04/ 01/15 – 03/31/20
Total Direct Costs: $3,124,495

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD
Title: "Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.
Source: Sandy Rollman Ovarian Cancer Foundation (SROCF) Fellowship
Date: 06/01/14 – 05/31/15
Total Direct Costs: $50,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD
Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.
Source: Ladies Auxiliary to the Veterans of Foreign Wars, Postdoctoral Cancer Research Fellowship
Date: 06/01/14 – 05/31/16
Total Direct Costs: $50,000

Role: Mentor, Percent Effort: 0%; PI: NM King, PhD
Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.
Source: Kaleidoscope of Hope Foundation, Young Investigator Award
Date: 04/01/14 – 03/31/15
Total Direct Costs: $50,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD
Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.
Source: Damon Runyon Cancer Research Foundation
Date: 07/01/14 – 06/30/17
Total Direct Costs: $158,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Chemoresistance Induces a Genotype Switch in Epithelial Ovarian Cancer Cells." The project was designed to determine whether development of chemoresistance in ovarian is attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.
Source: American Cancer Society Postdoctoral Fellowship
Date: 01/01/15 – 12/31/18
Total Direct Costs: $163,500

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD
Title: "Novel Biomarkers for the Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.
Source: American Association for Cancer Research
Date: 2015 – 2016
Total Direct Costs: $50,000

Role: Principal Investigator, Percent Effort: 30%
Title: "Postoperative Adhesion Development is Controlled by Mechanisms that Emanate from a Hypoxia-Induced Genotype Switch in Key Enzymes of Oxidative Stress." Identification of markers that are strongly associated with adhesions and in patients will contribute to both the delineation of mechanisms of adhesion development and serve as potential targets for therapy and intervention.
Source: NIH/NICHD, R01
Date: 07/01/15 – 06/30/20
Total Direct Costs: $1,921,633

Role: Principal Investigator, Percent Effort: 25%
Title: "Combination of Antioxidants Effectively Reduces Adhesion Development." The design of the project was to determine the effects of antioxidants on the prevention of postoperative adhesion development.
Source: NIH/NICHD, R03
Date: 07/01/15 – 06/30/17
Total Direct Costs: $153,314

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD
Title: "New Insights into the Pathogenesis of Ovarian Cancer." To identify keymarkers of oxidative stress that have the potential to serve as screening tools for ovarian cancer and may play a role in the acquisition of chemoresistance.
Source: Prevent Cancer Foundation
Date: 04/01/14 – 01/31/16
Total Direct Costs: $80,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: AO Awonuga, MD
Title: "Effects of Dietary Lycopene on Incidence and Severity of Postoperative Adhesions." The design of the project was to determine the effects of antioxidants on the prevention of postoperative adhesion development.
Source: NIH/NICHD, R03
Date: 09/01/14 – 08/31/16
Total Direct Costs: $152,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD

Title: "Novel Biomarkers for Detection of Early Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.
Source: Marsha Rivkin Center for Ovarian Cancer Research, Scientific Scholar Award Postdoctoral Fellowship
Date: 04/01/14 – 03/31/15
Total Direct Costs: $60,000

Role: Mentor; Percent Effort: 0%; Principal Investigator: J Belotte, MD
Title: "Catalase SNP as a Genetic Predictor for Epithelial Ovarian Cancer." The design of the project was to determine whether a SNP in the catalase gene could be utilized as a predictive marker for epithelial ovarian cancer.
Source: Marsha Rivkin Center for Ovarian Cancer Research, Scientific Scholar Award Postdoctoral Fellowship
Date: 04/01/14 – 03/31/15
Total Direct Costs: $60,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: NM King, PhD
Title: "Novel Biomarkers for the Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron could be utilized as biomarkers for the early detection of ovarian cancer.
Source: Hope Funds Cancer Research Postdoctoral Fellowship
Date: 2014 – 2016
Total Direct Costs: $100,000

Role: Principal Investigator
Title: "Chemoresistance in Ovarian Cancer Manifests a Genotype Switch in Oxidant Enzymes." The design of the project was designed to determine whether a genotype switch in key oxidant enzymes is induced in chemotherapy treated ovarian cancer cells and the subsequent effect of the enzymatic activity.
Source: Marsha Rivkin Center for Ovarian Cancer Research; Pilot Study
Date: 04/01/14 – 03/31/15
Total Direct Costs: $75,000

Role: Principal Investigator, Percent Effort: 30%; Co-Investigators: MP Diamond, MD, S Ghamande, PhD
Title: "Chemoresistance Induces a Genotype Switch in Redox Enzymes in Ovarian Cancer." The design of the project was to determine whether development of chemoresistance in ovarian cancer were attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.
Source: NIH/NICHD, R01
Date: 07/01/14 – 06/30/19
Total Direct Costs: $2,932,687

Role: Principal Investigator, Percent Effort: 25%
Title: "Chemoresistance in Ovarian Cancer is attributed to Enhanced Oxidative Stress." The design of the project was to determine whether development of chemoresistance in ovarian cancer were attributed to enhanced oxidative stress.
Source: NIH/NICHD, R03
Date: 07/01/14 – 06/30/16
Total Direct Costs: $152,000

Role: Principal Investigator, Percent Effort: 25%
Title: "Chemoresistance in Ovarian Cancer Manifests a Genotype Switch in Oxidant Enzymes." The design of the project was to determine whether development of chemoresistance in ovarian cancer were attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.
Source: NIH/NICHD, R03
Date: 07/01/14 – 06/30/16
Total Direct Costs: $152,000

Role: Mentor, Percent Effort: 0%; Principal Investigator: J Belotte, MD
Title: "Characterization of Epithelial Ovarian Cancer Stem Cells." The design of the project was to determine the role of pluripotency markers in epithelial ovarian cancer and the association with survival.
Source: NIH/NICHD, R03
Date: 09/30/14 – 06/30/16
Total Direct Costs: $152,000

Role: Mentor, Percent Effort: 0%; PI: J Belotte, MD
Title: "Catalase SNP as a Genetic Predictor for Epithelial Ovarian Cancer." The design of the project was to determine whether a SNP in the catalase gene could be utilized as a predictive marker for epithelial ovarian cancer.
Source: NIH/NICHD, R03
Date: 09/30/14 – 08/31/16
Total Direct Costs: $152,000

Role: Principal Investigator, Percent Effort: 20%
Title: "Innovative New Target for Ovarian Cancer Therapy." The project was designed to identify and test a target that cross-reacts with the CD11b antibody and determines its efficacy in killing both sensitive and chemoresistant ovarian cancer cells.
Source: NIH/NICHD, R21
Date: 12/31/16 – 11/30/18
Total Direct Costs: $275,000

Role: Principal Investigator
Title: "Redox Enzyme-Mediated Prosurvival of Chemoresistance in Ovarian Cancer." The design of the project was to determine whether development of chemoresistance in ovarian cancer were attributed to enhanced oxidative stress leading to a genotype switch in key oxidant and antioxidant enzymes.
Source: Ovarian Cancer Research Fund, Program Project Development Grant
Date: 201 6 – 2019
Total Direct Costs: $900,000

Role: Principal Investigator, Percent Effort: 20%
Title: "Novel Mechanisms of Apoptosis in Chemoresistant Ovarian Cancer Cells." The design of the project was to determine whether chemoresistance in ovarian cancer manifests decreased apoptosis through enhanced s-nitrosylation of caspase-3 mechanism, which could be reversed by DCA.
Source: NIH/NICHD, R21
Date: 12/01/16 – 11/30/18
Total Direct Costs: $275,000

Role: Mentor, Percent Effort 0%; Principal Investigator: NM King, PhD
Title: "Novel Biomarkers for Early Detection of Ovarian Cancer." The design of the project was to determine whether MPO and free iron can be utilized as biomarkers for the early detection of ovarian cancer.
Source: L'Oreal USA for Women in Science Fellowship
Date: 2016 – 2017
Total Direct Costs: $60,000

PATENTS:

COMPOSITIONS AND METHODS TO TREAT SOLID TUMORS
Publication Number: 20190309073
Abstract: Compositions and methods that utilize anti-CD11b antibodies, anti-CD18 antibodies, anti-myeloperoxidase (MPO) antibodies, anti-integrin alpha-V antibodies, anti-integrin beta-1 antibodies, Abciximab, neutrophil inhibitory factor (NIF) protein, and/or combinations thereof to treat solid tumor cancers are described.
Type: Application
Filed: May 20, 2019
Publication Date: October 10, 2019
Applicant: Wayne State University
Inventor: Ghassan M. Saed

METHOD OF PREVENTING ADHESIONS BY APOPTOSIS OF ADHESION PERITONEAL CELLS
Publication Number: 20040131600
Abstract: Methods for the prevention of adhesion formation and development involve the administration of therapeutic formulations to a patient which modulates the rate of apoptosis of adhesion fibroblast cells. The formulations preferably include Bax, Bax enhancers, such as p53, Bax agonists, Bcl-2 inhibitors and Bcl-2 antagonists. A method is also provided for determining the predisposition of a subject to adhesion formation by measuring the Bcl-2/Bax ratio at multiple sites within the subject.
Type: Application
Filed: March 1, 2004
Publication Date: July 8, 2004
Inventor: Ghassan M. Saed

MODEL FOR IN VITRO ADHESION DEVELOPMENT
Publication Number: 20040096817
Abstract: A biological model for the development of adhesions in vitro comprises a pair of opposed surfaces of tissue explants maintained in a culture media for a sufficient time and under conditions to permit the formation of adhesions. The model is useful for evaluating compounds and techniques for the prevention and remediation of adhesions, and for individualizing the therapeutic options for patients who may experience adhesions.
Type: Application
Filed: May 28, 2003
Publication Date: May 20, 2004
Applicant: Wayne State University
Inventor: Ghassan M. Saed

METHODS OF TREATING CANCER WITH CD 11B ANTIBODIES
International Publication Number: WO 2010/017083 AI
International Publication Date: February 11, 2010
Description of Patent: Methods and compositions for treating cancer with CDI Ib antibodies are disclosed. The antibodies may be WT.5 antibodies or compete with WT.5 antibodies, and induce apoptosis in SKOV, MDAH-2274, or BxPC-3 cells.
Inventor: Ghassan M. Saed

METHOD OF PREVENTING ADHESIONS WITH INTERFERON-GAMMA
International Publication Number: WO 02/072016 A3
International Publication Date: September 19, 2002
Description of Patent: Methods for the prevention of adhesion formation and development involve the administration of therapeutic formulations to a patient which include, as active ingredients, IFN-y or IFN-y enhancers. The IFN-y or IFN-y enhancers are preferably administered to fibrosis tissues in a subject prior to an event which induces adhesion formation, such as a surgical event.
Inventor: Ghassan M. Saed

METHODS FOR THE PREVENTION OF ADHESION FORMATION AND DEVELOPMENT
Patent Number: US2006025364
Application Number: US20050081278
Application Date: March 16, 2005
Publication Date: February 2, 2006
Description of Patent: Methods for the prevention of adhesion formation and development, and for the stimulation of fibrosis, involve the administration of therapeutic formulations to a patient containing inhibitors or stimulators to selected molecular adhesion markers. The molecular markers of the invention include Caspase 2, Caspase 3, Caspase 9, PPAR alpha, PPAR beta, PPAR gamma1, PPAR gamma2, and NF-kappa B.
Inventor: Ghassan M. Saed

PUBLICATIONS

Peer-Reviewed Publications

**Indicates student, trainee, or postdoctoral*

Reports of Original Work

1. Awonuga A.O., Chatzicharalampous C., Thakur M., Rambhatla R., Qadri F., Awonuga M., **Saed GM**., Diamond MP. Genetic and Epidemiological Similarities, and Differences Between Postoperative Intraperitoneal Adhesion Development and Other Benign Fibro-proliferative Disorders. Reprod. Sci. (2021). https://doi.org/10.1007/s43032-021-00726-9

2. Thakur M., Rambhatla A., Qadri F., Chatzicharalampous C., Awonuga M., **Saed GM**., Diamond MP., Awonuga, A.O. Is There a Genetic Predisposition to Postoperative Adhesion Development? *Reprod. Sci.* 28, 2076–2086 (2021). https://doi.org/10.1007/s43032-020-00356-7

3. Irene Peregrin-Alvarez, Nicole M Fletcher, **Ghassan M Saed**, Robert A Roman, Laura Detti. Anti-Müllerian Hormone (AMH) regulates BRCA1 and BRCA2 gene expression after ovarian cortex transplantation. Gynecol Endocrinol. 2020 Oct 21;1-4. PMID: 33084436

4. Laura Detti, Mustafa I Abuzeid, Irene Peregrin-Alvarez, Mary E Christiansen, Pouran Malekzadeh, Jennifer Sledge, **Ghassan M Saed**. Recombinant Anti-Müllerian Hormone (rAMH) for Stalling In Vitro Granulosa Cell Replication. Reprod. Sci. 2020 July2; (27) 1873–1878. Doi: 10.1007/s43032-020-00206-6

5. *Harper A, *Fletcher NM, *Fan R, Morris RT, **Saed GM.** Heat Shock Protein 60 (HSP60) Serves as A Potential Target for the Sensitizatioin of Chemoresistant Ovarian Cancer Cells. Reprod Sci. 2020 Apr27;(4):1030-1036. doi: 10.1007/s43032-019-00089-2. Epub 2020 Mar 2.PMID: 32124395

6. *Fletcher NM, *Harper A, *Memaj I, *Fan R, Morris RT, **Saed GM**. Molecular basis supporting the association of talcum powder use with increased risk of ovarian cancer. Reproductive Sciences. 2019 Dec; 26(12) P1603-1612

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Role: Mentor and collaborator

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Role: Mentor and collaborator

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Role: Mentor and collaborator

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Role: Mentor, advisor, collaborator, assisted with research

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Role: Mentor, collaborator, assisted with experimental format

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Role: Mentor, collaborator, assisted with writing of article

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Role: Mentor, collaborator, assisted with manuscript writing

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Role: Collaborator

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Role: Collaborator and assisted with manuscript writing

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Role: Collaborator and mentor

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Role: Collaborator

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Role: Mentor and collaborator

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Role: Mentor and collaborator

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Role: Mentor and collaborator

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Role: Mentor and collaborator

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Role: Mentor, collaborator, assisted with writing of article

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Role: Mentor and collaborator; assisted with experimental design

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Role: Mentor and collaborator

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Role: Mentor and collaborator

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Role: Mentor and collaborator

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Role: Mentor and collaborator

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Role: Collaborator and mentor; assisted with experimental design

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Role: Collaborator, mentor and assisted with research protocol

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Role: Collaborator and mentor

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Role: Mentor and collaborator, assisted with research protocol and writing of article

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Role: Collaborator

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Role: Mentor, collaborator

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Role: Mentor and collaborator

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Role: Mentor and collaborator

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Role: Mentor, collaborator, assisted with experimental design

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Role: Collaborator and assisted with experimental design

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Role: Mentor and collaborator, assisted with manuscript writing and experimental design

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Role: Mentor and collaborator, assisted with experimental design

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Role: Collaborator

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Role: Mentor and collaborator

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Role: Mentor, collaborator, assisted with experimentation and writing of manuscript
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Role: Mentor and collaborator

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Role: Mentor and collaborator, assisted with experimental design and manuscript writing

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235. **Saed GM**, Fivenson D. The effect of PUVA treatment on HUT78 cell differential gene expression. Journal of Investigative Dermatology 106:906, 1996.

236. Hou Z, **Saed GM**, Fivenson D, Ladin D. Hypoxia does not alter cytokine production by keloid fibroblasts. Journal of Investigative Dermatology 104:669, 1995.

237. Ladin D, Hou Z, **Saed GM**, Fivenson D. Hypoxia upregulates TGF-β1 and its receptor expression by monocytes but its effects are blocked by chronic wound fluid. Journal of Investigative Dermatology 104: 590, 1995.

238. Fivenson D, Elkheimi M, **Saed GM**, Nickoloff B. Modulation of IL-10 expression in HUT78 cells: Insights into the pathobiology and treatment of CTCL. Journal of Investigative Dermatology 104: 648, 1995.

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240. **Saed GM**, Labelle D, Fivenson D. Detection of differentially displayed cDNA fragments in normal vs Sezary syndrome leukocytes. Journal of Investigative Dermatology 104: 673, 1995.

241. **Saed GM**, Fivenson DP, Nickoloff BJ. Quantitative PCR analysis of Th-1 cytokines in HUT78 cells after exposure to PUVA in vitro. Journal of Investigative Dermatology 102: 585, 1994.

242. Fivenson DP, **Saed GM**. Augmentation of Th-1 cytokines in the peripheral blood of Sezary syndrome patients after treatment with ECCP. Journal of Investigative Dermatology 102: 586, 1994.

243. Fivenson DP, **Saed GM**, Nickoloff BJ. Modulation of IL-10 expression in HUT78 cells: insights into the pathobiology and treatment of CTCL. Clinical Research 42: 232, 1994.

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245. **Saed GM**, Fivenson DP. Augmentation of Th-1 cytokines in the peripheral blood of SZ patients upon treatment with extracorporeal photopheresis. Clinical Research 41: 664, 1993.

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247. **Saed GM**, Fivenson DP. Detection of T-cell clonality in mycosis fungoides by PCR-metaphore agarose analysis of T-cell receptor-γ. Clinical Research 41: 459, 1993.

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249. Dillon M, **Saed G**, Fivenson D. PCR analysis of Borrelia burgdorferi in skin lesions of morphea, Scleroderma, and Lichen Sclerosus et Atrophicans. Clinical Research 41: 427, 1993.

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Non Peer-Reviewed Publications

Other (On-Line Publications) * Indicates student, trainee, or postdoctoral

1. *Nusrat O, *Belotte J, *Fletcher NM, *Memaj I, *Saed MG, Diamond MP, **Saed GM**. The role of angiogenesis in the persistence of chemoresistance in epithelial ovarian cancer. www.OncToday.com, Beyond the Abstract, June 21, 2016.

2. *Belotte J, *Fletcher NM, *Alexis M, Morris RT, Munkarah AR, Diamond MP, **Saed GM**. Sox2 gene amplification significantly impacts overall survival in serous epithelial ovarian cancer. Global Medical Discovery Series (*Key Scientific Article Contributing to Excellence in Biomedical Research*), summer issue 2015.

3. *Fletcher NM, *Saed MG, Abu-Soud HM, Al-Hendy A, Diamond MP, **Saed GM**. Uterine fibroids are characterized by an impaired antioxidant cellular system: potential role of hypoxia in the pathophysiology of uterine fibroids. Featured article. MDLinx.com/ obstetrics-gynecology/ news-article, November 2013.

PRESENTATIONS

Podium Presentations (Referred)

1. *Cyclophosphamide and Its Metabolite Impact on Fertilization through Mitochondrial Dysfunction*. 65th Annual Meeting of the Pacific Coast Reproductive Society, Indian Wells, CA, March 2017.

2. *Novel Target for Ovarian Cancer Immunotherapy*. 48th Annual Meeting of the Society of Gynecologic Oncology's Women's Cancer, National Harbor, MD, March 2017.

3. *Targeting Integrin αV/β1 Receptor Manifests Intriguing Anti-Tumor Effects in Sensitive and Chemoresistant Ovarian Cancer Cells: Potential Therapeutic Target*. 64th Annual Scientific Meeting of the Society for Reproductive Investigation, Orlando, FL, March 2017.

4. *Abciximab Manifests Striking Anti-Tumor Effects in Sensitive and Chemoresistant Ovarian Cancer Cells. Ghassan M. Saed PhD, Nicole M. Fletcher PhD, Ira Memaj BS Wayne State University School, Department of Obstetrics and Gynecology, Detroit, MI.* Reproductive Sciences Retreat and MARTS 2017

5. *Human Adhesion Fibroblasts are Characterized by Reduction in the level* of *Pluripotency Markers as Compared to Normal Peritoneal Fibroblasts*. 72nd Annual Meeting of the American Society for Reproductive Medicine, Salt Lake City, UT, October 2016.

6. *Anti-Mullerian Hormone (AMH) for Prevention of Tissue Activation after Vitrified/Thawed Ovarian Cortex Xenotransplantation*. 72nd Annual Meeting of the American Society for Reproductive Medicine, Salt Lake City, UT, October 2016.

7. *Dichloroacetate Induces Apoptosis of Uterine Leiomyoma Cells Through A Mechanism Involving Modulation of Oxidative Stress*. 63rd Annual Meeting of the Society for Reproductive Investigation, Montreal, Quebec, Canada, March 2016.

8. *Chemoresistance in Epithelial Ovarian Cancer Cells is Controlled by Mechanisms Emanating from Chemotherapy-Induced Genotype Switch in Glutathione Peroxidase, Through the Up-Regulation of Cytidine Deminase*. 62nd Annual Meeting of the Society for Reproductive Investigation, San Francisco, CA, March 2015.

9. *Elevated Serum Anti-Müllerian Hormone (AMH) Stalls Ovarian Follicle Development by Downregulating FSH- and LH-Receptors and Inhibin-B Production*. Proceedings of the 71st Annual Meeting of the American Society for Reproductive Medicine, Baltimore, MD, October 2015.

10. *Hypochlorous Acid Reversibly Inhibits Caspase-3: A Potential Regulator of Apoptosis*. Joint Meeting of the 22nd Society for Redox Biology and Medicine (SFRBM) and 17th Society for Free Radical Research International (SFRRI), Boston, MA, November 2015.

11. *The In-Vivo Effects of Superoxide Dismutase on the Incidence and Severity of Post-Operative Adhesion Development*. 70th Annual Meeting of the American Society for Reproductive Medicine, Honolulu, HI, October 2014.

12. *Superoxide Dismutase Significantly Delayed the Development of Cisplatin Resistance in Epithelial Ovarian Cancer Cells*. American Association for Cancer Research's Precision Medicine Series: Drug Sensitivity and Resistance. Improving Cancer Therapy Special Conference, Orlando, FL, June 2014.

13. *Chemoresistant Ovarian Cancer Cells Manifest Lower Vascular Endothelial Growth Factor and Hypoxia Induced Factor-1α: A Potential Survival Mechanism*. American Association for Cancer Research's Precision Medicine Series: Drug Sensitivity and Resistance. Improving Cancer Therapy Special Conference, Orlando, FL, June 2014.

14. *Dicholoroacetate Increases Sensitivity to Chemotherapy by Modulation of Antioxidants in Epithelial Ovarian Cancer*. 61st Annual Meeting of the Society for Gynecologic Investigation, Florence, Italy, March 2014.

15. *Catalase and NADPH Oxidase Single Nucleotide Polymorphisms Are Associated with Increased Risk and Serve As Potential Targets for Breast and Ovarian Cancers*. 104th Annual Meeting of the American Association for Cancer Research, Washington, DC, April 2013.

16. *The Role of Oxidative Stress in the Development of Cisplatin Resistance in Epithelial Ovarian Cancer*. Poster session B. Advances in Ovarian Cancer Research: From Concept to Clinic. American Association for Cancer Research, Miami, FL, September 2013.

17. *Catalase and NADPH Oxidase Single Nucleotide Polymorphisms Are Associated with Increased Risk and Serve As Potential Targets for Breast and Ovarian Cancers*. 104th Annual Meeting of the American Association for Cancer Research, Washington, DC, April 2013.

18. *Endometrial Insulin Pathway during Ovarian Stimulation for Assisted Reproductive Technology (ART)*. 68th Annual Meeting of the American Society for Reproductive Medicine, San Diego, CA, October 2012.

19. *NADPH Oxidase p22-Phox Gene Polymorphism in Women is Associated with the Development of Postoperative Adhesions*. 59th Annual Meeting of the Society for Gynecologic Investigation, San Diego, CA, March 2012.

20. *Metabolism and Oxidative Stress: Integral Role in Regulation of the Adhesion Phenotype*. 58th Annual Meeting of the Society for Gynecologic Investigation, Miami Beach, FL, March 2011.

21. *Mass Spectrometric Identification of HOCl-Mediated Heme Degradation Products of Hemoglobin*. 59th ASMS Conference on Mass Spectrometry, Denver, CO, 2011.

22. *Inhibition of NADPH Oxidative Reductase Promotes Apoptosis in Epithelial Ovarian Cancer Cells*. 39th Annual Meeting of the Global Congress of Minimally Invasive Gynecology AAGL, Las Vegas, NV, November 2010.

23. *Reaction of Hemoglobin and Red Blood Cells with Hypochlorous Acid and Mechanism of Heme Destruction and Free Iron Release*. 17th Annual Meeting of the Society for Free Radical Biology and Medicine, Orlando, FL, November 2010.

24. *Liquid Chromatography Atmospheric Pressure Ionization Tandem: Mass Spectrometry Identifies Novel Hypochlorous Acid Reaction Products of Lycopene*. 58th Annual Meeting of the American Society of Mass Spectrometry, Salt Lake City, UT, May 2010.

25. *Role of Polychlorinated Biphenyls Enhancement of Lipid Peroxidation in Human Normal Peritoneal and Adhesion Fibroblasts*. 38th Annual Meeting of Global Congress of Minimally Invasive Gynecology AAGL, Orlando, FL, November 2009.

26. *Hydrogen Peroxide Bioavailability Determines the Sensitivity of Human Normal Peritoneal and Adhesion Fibroblasts to Hypoxia-Induced Lipid Peroxidation*. 38th Annual Meeting of Global Congress of Minimally Invasive Gynecology AAGL, Orlando, FL, November 2009.

27. *S-Nitrosylation of Caspase-3 Is the Mechanism by Which Adhesion Fibroblasts Manifest Lower Apoptosis*. 36th Annual Meeting of the American Association of

Gynecologic Laparoscopists, Global Congress of Minimally Invasive Gynecology, Washington, DC, November 2007.

28. *Generation of Superoxide by Inducible Nitric Oxide Synthase in L-Arginine Deficient Fibroblasts Established From Human Adhesion Tissues*. 36th AAGL Annual Meeting, Global Congress of Minimally Invasive Gynecology, Washington, DC, November 2007.

29. *Hypoxia Stimulation of Expression of Type I Collagen and Fibronectin in Human Peritoneal and Adhesion Fibroblasts: Blockage by Interferon Gamma*. 36th AAGL Annual Meeting, Global Congress of Minimally Invasive Gynecology, Washington, DC, November 2007.

30. *Superoxide Induces the Adhesion Phenotype: Role of Hypoxia in the Pathogenesis of the Adhesion Development*. Global Congress of Minimally Invasive Gynecology, 36th Annual Meeting of the American Association of Gynecologic Laparoscopists, Washington, DC, November 2007.

31. *Nitric Oxide Synthase Isoforms are Differentially Expressed in Fibroblasts Isolated from Human Normal Peritoneum and Adhesion Tissues*. 63rd Annual Meeting of the American Society for Reproductive Medicine, Washington, DC, October 2007.

32. *Regulation of the Expression of INOS, COX-2, and VEGF in Postoperative Adhesions*. 62nd Annual Meeting of the American Society for Reproductive Medicine, New Orleans, LA, October 2006.

33. *Omega-3 Fatty Acid Prevents and Mitigates the Adhesion Phenotype in Normal Human Peritoneal and Adhesion Fibroblasts*. 62nd Annual Meeting of the American Society for Reproductive Medicine, New Orleans, LA, October 2006.

34. *IL6 Expression in Human Normal Peritoneal and Adhesion Fibroblasts: Regulation by Hypoxia*. 62nd Annual Meeting of the American Society for Reproductive Medicine, New Orleans, LA, October 2006.

35. *The Cross-Talk between Myeloperoxidase and Inducible Nitric Oxide Synthase in Post-operative Adhesions*. 62nd Annual Meeting of the American Society for Reproductive Medicine, New Orleans, LA, October 2006.

36. *TNF-Alpha Expression in Human Normal Peritoneal and Adhesion Fibroblasts: Regulation by Hypoxia*. 62nd Annual Meeting of the American Society for Reproductive Medicine, New Orleans, LA, October 2006.

37. *L-Arginine Deficiency in Fibroblasts Established from Human Adhesion Tissues Leads to the Generation of Superoxide by Inducible Nitric Oxide Synthase*. 53rd Annual Meeting of the Society for Gynecologic Investigation, Toronto, Ontario, Canada, March 2006.

38. *Regulation of Inducible Nitric Oxide Synthase in Post-Operative Adhesions*. 34th Annual Meeting of the American Association of Gynecologic Laparoscopists, Chicago, IL, November 2005.

39. *Cyclooxygenase-2 Inhibitors Enhance Apoptosis of Adhesion Fibroblasts*. 34th Annual Meeting of the American Association of Gynecologic Laparoscopists, Chicago, IL, November 2005.

40. *The Effects of Estradiol on the Expression of Estrogen, Progesterone, Androgen, and Prolactin Receptors in Human Peritoneal Fibroblasts*. 61st Annual Meeting of the American Society for Reproductive Medicine and the 1st Annual Meeting of the Canadian Fertility and Andrology Society, Palais des Congres, Montreal, Quebec, Canada, October 2005.

41. *Possible Role of Natural Immune Response against Fibroblasts in the Development of Post-Operative Adhesions*. 61st Annual Meeting of the American Society for Reproductive Medicine and the 51st Annual Meeting of the Canadian Fertility and Andrology Society, Palais des Congres, Montreal, Quebec, Canada, October 2005.

42. *Knockout of Inducible Nitric Oxide Expression Significantly Reduces the Expression of Type I Collagen and Transforming Growth Factor-β1 in Human Peritoneal and Adhesion Fibroblasts*. 61st Annual Meeting of the American Society for Reproductive Medicine and the 51st Annual Meeting of the Canadian Fertility and Andrology Society, Palais des Congres, Montreal, Quebec, Canada, October 2005. **Prize Paper Candidate**

43. *Regulation of Inducible Nitric Oxide Synthase in Post-Operative Adhesions*. 52nd Annual Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2005.

44. *Differential Expression of Myeloperoxidase (MPO) in Fibroblasts Isolated from Normal Peritoneal and Adhesion Tissues*. 4th International Peroxidase Meeting Joint with the 10th Myeloperoxidase Meeting, Shimokyo-Ku, Kyoto City, Japan, October 2004.

45. *Fibroblasts Isolated from Normal Peritoneal and Adhesion Tissues Differentially Express Myeloperoxidase (MPO)*. 60th Annual Meeting of the American Society for Reproductive Medicine, Philadelphia, PA, October 2004.

46. *Hypoxia Up-Regulates Cyclooxygenase-2 and Prostaglandin E_2 Levels in Human Peritoneal Fibroblasts*. 60th Annual Meeting of the American Society for Reproductive Medicine, Philadelphia, PA, October 2004.

47. *Dichloroacetate Inhibition of Angiogenesis Caused by Hypoxia Treatment of Normal Peritoneal and Adhesion Fibroblasts in Human Umbilical Vein Endothelial Cells*. 60th Annual Meeting of the American Society for Reproductive Medicine, Philadelphia, PA, October 2004.

48. *Dichloroacetate Significantly Increase the Expression of the Transcription Nuclear Factor Kappa-β in Fibroblasts of Human Adhesion Tissues*. 51st Annual Scientific Meeting of the Society for Gynecologic Investigation, Houston, TX, March 2004.

49. *Stimulation of Expression of Vascular Endothelial Growth Factor by Hypoxia from Fibroblasts Isolated from Normal Peritoneum and Adhesion Tissues*. 32nd Annual Meeting of The American Association of Gynecologic Laparoscopists, Las Vegas, NV, November 2003.

50. *Inhibition of Nitric Oxide Production by N-Nitro-L-Arginine Methyl Ester Increased the Expression of Type I Collagen in Human Peritoneal Fibroblasts*. 59th Annual Meeting of American Society for Reproductive Medicine, San Antonio, TX, October 2003.

51. *Apoptosis of Human Peritoneal and Adhesion Fibroblasts After Hypoxia: Role of Inducible Nitric Oxide Synthase*. 59th Annual Meeting of American Society for Reproductive Medicine, San Antonio, TX, October 2003.

52. *Inhibition of Cyclooxygenase-2 in Fibroblasts Isolated from Normal Peritoneum and Adhesion Tissues Decreases the Expression of Hypoxia Inducible Factor-1 Alpha*. 50th Annual Scientific Meeting of the Society for Gynecologic Investigation, Washington, DC, March 2003.

53. *Tissue Plasminogen Activator/Plasminogen Activator Inhibitor-1 (tPA/PAI-1) Modulation by Tisseel*. 50th Annual Scientific Meeting of the Society for Gynecologic Investigation, Washington, DC, March 2003.

54. *Hypoxia Increases the Expression of Vascular Endothelial Growth Factor in Fibroblasts Isolated From Human Normal Peritoneum and Adhesion Tissues*. 50th Annual Scientific Meeting of the Society for Gynecologic Investigation, Washington, DC, March 2003.

55. *Dichloroacetate Significantly Reduces the Expression of Vascular Endothelial Growth Factor in Fibroblasts of Human Adhesion Tissues*. 50th Annual Scientific Meeting of the Society for Gynecologic Investigation, Washington, DC, March 2003.

56. *Transforming Growth Factor-Beta 1 (TGF-β1) and Extracellular Matrix Production by Human Peritoneal Mesothelial Cells: Effect of Tisseel® Fibrin Sealant)*. 50th Annual Scientific Meeting of the Society for Gynecologic Investigation, Washington, DC, March 2003.

57. *Cyclooxygenase-2 Inhibition Decreases the Expression of Vascular Endothelial Growth Factor from Fibroblasts Isolated from Normal Peritoneum and Adhesion Tissues*. 50th Annual Scientific Meeting of the Society for Gynecologic Investigation, Washington, DC, March 2003.

58. *Elevation of Type I Collagen mRNA in Peritoneal Adhesions*. 31st Annual Meeting of the American Association of Gynecologic Laparoscopists, Miami, FL, November 2002.

59. *Cyclooxygenase-2 Expression in Human Fibroblasts Isolated from Adhesions But Not from Normal Peritoneal Tissues*. 31st Annual Meeting of the American Association of Gynecologic Laparoscopists, Miami, FL, November 2002.

60. *Existence of p53 Expression in Human Fibroblasts Isolated from Adhesions, But Not from Normal Peritoneal Tissues*. 31st Annual Meeting of the American Association of Gynecologic Laparoscopists, Miami, FL, November 2002.

61. *Matrix Metalloproteinase (MMP-1, MMP-2), and Tissue Inhibitor for Metalloproteinase (TIMP-1) Expression by Human Peritoneal Mesothelial Cells: Effect of Fibrin Sealant*. 58th Annual Meeting of the American Society for Reproductive Medicine, Seattle, WA, October 2002.

62. *Dichloroacetate (DCA) Significantly Increases the Expression of Inducible Nitric Oxide Synthase (INOS) in Human Fibroblasts of Adhesion Tissues, But Not In Normal Peritoneum*. 58th Annual Meeting of the Society for Reproductive Medicine, Seattle, WA, October 2002.

63. *Seprafilm (Modified Hyaluronic Acid Carboxymethylcellulose) Acts as a Mechanical Barrier*. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.

64. *Inhibition of Cyclooxygenase-2 in Human Adhesion Fibroblasts Reduces the Expression of MMP-1 and TIMP-1*. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.

65. *Inhibition of Cyclooxygenase-2 in Human Adhesion Fibroblasts Reduces the Expression of Transforming Growth Factor Beta-1*. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.

66. *Adhesion Phenotype: Cyclooxygenase-2 is Expressed in Fibroblasts Isolated From Adhesions, But Not From Normal Peritoneal Tissues*. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.

67. *Reduction of the Expression of Type I and III Collagens in Human Adhesion Fibroblasts, But Not in Normal Peritoneal Fibroblasts by the Inhibition of Cyclooxygenase-2*. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.

68. *Dichloroacetate Significantly Reduces the Expression of Cyclooxygenase-2 in Human Fibroblasts of Adhesion Tissues*. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.

69. *Adhesion Phenotype: p53 is Expressed in Fibroblasts Isolated From Adhesions But Not From Normal Peritoneal Tissues*. 49th Scientific Meeting of the Society for Gynecologic Investigation, Los Angeles, CA, March 2002.

70. *Metabolic Regulation of Collagen I in Fibroblasts Isolated from Normal Peritoneum and Adhesions by Dichloroacetic Acid (DCA)*. 28th Scientific Meeting of Gynecologic Surgeons, Dallas, TX, March 2002.

71. *An Adhesion Promoting Phenotype: Implications for Postoperative Adhesion Development*. 30th Annual Meeting American Association of Gynecologic Laparoscopists, Global Congress of Gynecologic Endoscopy, San Francisco, CA, November 2001.

72. *Differences in the Rate of Apoptosis Following Hypoxia in Human Peritoneal and Adhesion Fibroblasts*. 30th Annual Meeting American Association of Gynecologic Laparoscopists, Global Congress of Gynecologic Endoscopy, San Francisco, CA, November 2001.

73. *Modulation of the BCL-2/BAX Ratio by IFN-GAMMA and Hypoxia in Human Peritoneal and Adhesion Fibroblasts*. 57th Annual Meeting of the American Society for Reproductive Medicine, Orlando, FL, October 2001.

74. *Significance of the Effect of Hypoxia on the Rate of Apoptosis of Human Peritoneal and Adhesion Fibroblasts for Postoperative Adhesion Development*. 57th Annual Meeting of the American Society for Reproductive Medicine, Orlando, FL, October 2001.

75. *Prostaglandin E_2 Stimulates Proliferation and Reduces Apoptosis in Epithelial Ovarian Cancer Cell Lines*. 48th Annual Meeting of the Society for Gynecologic Investigation, Toronto, Canada, March 2001.

76. *Differential Modulation of BCL-2/BAX Ratio by Hypoxia in Peritoneal and Adhesion Fibroblasts Cultured from the Same Patient*. 48th Annual Meeting of the Society for Gynecologic Investigation, Toronto, Canada, March 2001.

77. *Interferon Gamma Blocks the Stimulating Effect of Hypoxia on the Expression of Type I Collagen and Fibronectin in Human Peritoneal and Adhesion Fibroblasts*. 48th Annual Meeting of the Society for Gynecologic Investigation, Toronto, Canada, March 2001.

78. *The Effect of Interferon Gamma and Hypoxia on the Expression of TGF-β Isoforms in Human Peritoneal and Adhesion Fibroblasts*. 48th Annual Meeting of the Society for Gynecologic Investigation, Toronto, Canada, March 2001.

79. *The Effect of Normoxia after Hypoxia Treatment of the Expression of Type I Collagen and TGF-β1 in Human Peritoneal Fibroblasts: Implications for Postoperative Adhesion Development*. 48th Annual Meeting of the Society for Gynecologic Investigation, Toronto, Canada, March 2001.

80. *Modulation of the BCL-2/BAX Ratio by IFN-γ and Hypoxia in Human Peritoneal and Adhesion Fibroblasts*. 32nd Annual Meeting of the Society of Gynecologic Oncologists, Nashville, TN, February 2001.

81. *Prostaglandin in Induced COX-2 Expression and Reduced Apopotosis in Epithelial Ovarian Cancer Cells*. 32nd Annual Meeting of the Society of Gynecologic Oncologists, Nashville, TN, February 2001.

82. *The Effect of Hypoxia on the Expression of HIF-1β, BAX, and BCL-2 in the Epithelial Ovarian Cancer Cell Line MADH2774*. 32nd Annual Meeting of the Society of Gynecologic Oncologists, Nashville, TN, February 2001.

83. *Induction of Cyclooxygenase-2 by Prostaglandin E_2 in Human Ovarian Cancer Cell Lines*. 53rd Congress of the DGGG, German Society of Gynecology and Obstetrics eV, Munich, Germany, June 2000.

84. *Type I Collagen Production by Human Peritoneal Fibroblasts in Response to Hypoxia and/or Transforming Growth Factor-Beta 1 (TGF-β1) Treatments*. 47th Annual Meeting of the Society for Gynecologic Investigation, SGI 2000-A Millennial Milestone in Reproductive Sciences: Celebrating the Promise, Chicago, IL, March 2000.

85. *The Effect of Hypoxia on TGF-β1 on the Expression of Cellular Fibronectin in Human Peritoneal Fibroblast Cells in Culture*. 47th Annual Meeting of the Society for Gynecologic Investigation, SGI 2000-A Millennial Milestone in Reproductive Sciences: Celebrating the Promise, Chicago, IL, March 2000.

86. *Type I Collagen Expression in Adhesion and Normal Peritoneal Tissues*. 47th Annual Meeting of the Society for Gynecologic Investigation, SGI 2000-A Millennial Milestone in Reproductive Sciences: Celebrating the Promise, Chicago, IL, March 2000.

87. *Vascular Endothelial Growth Factor (VEGF) Levels Are Elevated in Adhesion Tissue in Humans*. Annual Meeting of the American Association of Gynecologic Laparoscopists, Las Vegas, NV, November 1999.

88. *Basics of Cutaneous Wound Repair*. 4th International Conference on Postoperative Healing and Adhesions, Fort Lauderdale, FL, October 1999.

89. *The Role of Extracellular Matrix in the Formation of Postoperative Adhesion*. 4th International Conference on Postoperative Healing and Adhesions, Fort Lauderdale, FL, October 1999.

90. *The Effect of Hypoxia and TGF-β1 on the Expression of Tissue Inhibitors of Metalloproteinases (TIMP-1) in Human Peritoneal Mesothelial Cells*. Joint meeting of the Canadian Fertility Society and the American Society for Reproductive Medicine, Toronto, Ontario, Canada, September 1999.

91. *Collagen Type I and Type III Production by Human Mesothelial Cells in Response to Hypoxia and/or TGF-β1 Treatments*. Annual Meeting of the Society for Gynecologic Investigation, Atlanta, GA, March 1999.

92. *The Role of Apoptosis and p53 in the Pathogenesis of Keloids*. Journal of Investigative Dermatology 110: 597, 1998.

93. *Apoptosis Modulation in the Response of CTCL to PUVA*. Journal of Investigative Dermatology 110: 698, 1998.

94. *Apoptosis Dysregulation in Keloid Fibroblasts*. Journal of Investigative Dermatology 110:653, 1998.

95. *Apoptosis Regulation in the Pathogenesis of Cutaneous T-Cell Lymphoma (CTCL)*. Journal of Investigative Dermatology 108:610, 1997.

96. *The Effect of PUVA Treatment on HUT78 Cell Differential Gene Expression*. Journal of Investigative Dermatology 106:906, 1996.

97. *Detection of Differentially Displayed cDNA Fragments in Normal vs Sezary Syndrome Leukocytes*. Journal of Investigative Dermatology 104: 673, 1995.

98. *Quantitative PCR Analysis of Th-1 Cytokines in HUT78 Cells after Exposure to PUVA In Vitro*. Journal of Investigative Dermatology 102: 585, 1994.

99. *Augmentation of Th-1 Cytokines in the Peripheral Blood of Sezary Syndrome Patients after Treatment with ECCP*. Journal of Investigative Dermatology 102:586, 1994.

100. *Augmentation of Th-1 Cytokines in the Peripheral Blood of SZ Patients Upon Treatment with Extracorporeal Photopheresis*. Clinical Research 41:664, 1993.

101. *Detection of T-Cell Clonality in Mycosis Fungoides by PCR-Metaphore Agarose Analysis of T-Cell Receptor-γ*. Clinical Research 41:459, 1993.

102. *Mycosis Fungoides and Psoriasis Exhibit a Th1 Type Cell Mediated Response While Sezary Syndrome Expresses A Th2 Type Response*. Clinical Research 40:730, 1992.

103. *T-Cell Receptor Gene Conservation and Rearranged Clones in Canine Mycosis Fungoides*. Clinical Research 40:505, 1992.

Poster Presentations (Referred)

1. Fletcher NM, Memaj I, Diamond MP, Morris RT, **Saed GM.** Heat Shock Protein 60 (HSP60) Serves as a Potential Target for the Sensitization of Chemoresistant Ovarian Cancer Cells. 49th Society of Gynecologic Oncology Annual Meeting on Women's Cancer, New Orleans, LA, March 24-27, 2018. Gynecologic Oncology Supplement, 2018.

2. Fletcher NM, Memaj I, Diamond MP, Morris RT, **Saed GM.** Targeting Myeloperoxidase Enhances Apoptosis in Chemoresistant Epithelial Ovarian Cancer Cells by Reversing S-Nitrosylation of Caspase-3. 49th Society of Gynecologic Oncology Annual Meeting on Women's Cancer, New Orleans, LA, March 2018. Gynecologic Oncology Supplement, 2018.

3. Fletcher NM, Aownuga AO, Memaj M, Diamond MP, Al-Hendy AA, **Saed GM**. A Novel Role for the Interaction of Myeloperoxidase and CD11b in Leiomyoma Cells. 65th Annual Scientific Meeting for the Society for Reproductive Investigation, San Diego, CA, March 2018. Session Gynecology Proceedings: S-116, 2018.

4. Fletcher NM, Memaj I, **Saed GM.** Talcum Powder Enhances Oxidative Stress in Ovarian Cancer Cells. 65th Annual Scientific Meeting for the Society for Reproductive Investigation, San Diego, CA, March 2018. Session Gynecologic Oncology Proceedings: F-098, 2018.

5. Robertson L, Fletcher NM, **Saed GM**. L-Alanyl-L-Glutamine Attenuates the Levels of Adhesion Phenotype Markers in Normal Fibroblasts Isolated From Human Peritoneum Under Hypoxic Conditions. 65th Annual Scientific Meeting for the Society for Reproductive Investigation, San Diego, CA, March 2018. Session Gynecology Proceedings: F-102, 2018.

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22. **Saed GM**. Investigation of the role of oxidative stress in the pathophysiology of gynecologic fibrotic disorders including postoperative adhesions, fibroids, and endometriosis as well as ovarian cancer. 3rd Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2013. Program and Abstracts: #20, 2013.

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39. Maitra D, Abdulhamid I, **Saed GM**, Diamond MP, Pennathur S, Abu-Soud HM. Fluorescent heme degradation products in sickle cell disease: role of hypochlorous acid in hemoglobin destruction. 2nd Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2012. Programs and Abstracts: #16, 2012.

40. **Saed GM**. Investigation of the role of oxidative stress in the pathophysiology of gynecologic fibrotic disorders including postoperative adhesions, fibroids, and endometriosis as well as ovarian cancer. 2nd Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2012. Program and Abstracts: #9, 2012.

41. Shavell VI, Fletcher NM, Jiang ZL, **Saed GM**, Diamond MP. Coupling oxidative phosphorylation with 2,4-dinitrophenol promotes development of the adhesion phenotype. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011. Program and Abstracts: #26, 2011.

42. **Saed GM**. The role of oxidative stress in the pathophysiology of gynecologic fibrotic disorders including postoperative adhesions, fibroids, and endometriosis, as well as ovarian cancer. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011. Program and Abstracts: #24, 2011.

43. Maitra D, Shaeib F, Diamond MP, **Saed GM**, Abu-Soud HM. Melatonin can attenuate HOCl mediated hemolysis, free iron release and heme degradation from hemoglobin. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011. Program and Abstracts: #19, 2011.

44. Maitra D, Byun J, Andreana PR, Abdulhamid I, Diamond MP, **Saed GM**, Pennathur S, Abu-Soud HM. Reaction of hemoglobin with HOCl: possible link between free iron accumulation and oxidative stress. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011. Program and Abstracts: #18, 2011.

45. Fletcher NM, Jiang ZL, Levin NK, Abu-Soud HM, Munkarah AR, Tainsky MA, Diamond MP, **Saed GM**. Positive correlation between serum myeloperoxidase and free iron levels with stage of ovarian cancer: potential biomarkers for early detection and prognosis of ovarian cancer. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011. Program and Abstracts: #13, 2011.

46. Banerjee J, Maitra D, Shaeib F, **Saed GM**, Diamond MP, Abu-Soud HM. Role of melatonin in preventing hypochlorous acid induced alterations in microtubule and chromosomal structure in metaphase-II mouse oocytes *in vitro*. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011. Program and Abstracts: #7, 2011.

47. Diamond MP, **Saed GM**. Reduction of postoperative adhesions. Catalyzing Collaboration between Industry and Academic in the Life Sciences – Women's Health Medicine: Part I, Therapeutic Strategies Meeting, Illinois Science and Technology Park, Skokie, IL, June 2007. Proceedings 2007.

Invited Lectures/Presentations

International/National

1. *Targeting Integrin αV/β1 Receptor Manifests Intriguing Anti-Tumor Effects in Sensitive and Chemoresistant Ovarian Cancer Cells: Potential Therapeutic Target*. 64th Annual Scientific Meeting of the Society for Reproductive Investigation, Orlando, FL, March 2017.

2. *The Role of Oxidative Stress in the Pathogenesis of Ovarian Cancer*. University of Jordan, Amman, Jordan, July 2017.

3. *Novel Innovative Targets for Ovarian Cancer Therapy*. King Hussein Cancer Center, Amman, Jordan, July 2017.

4. *The Role of Oxidative Stress in the Pathogenesis of Ovarian Cancer*. King Hussein Cancer Center, Amman, Jordan, November 2016.

5. *The Role of Oxidative Stress in the Pathogenesis of Ovarian Cancer*. University of Jordan, Amman, Jordan, November 2016.

6. *New Insights for Ovarian Cancer Screening*. 4th International Conference of the Jordanian Society of Pathology and Laboratory Medicine. In collaboration with the Arabic Division of the International Academy of Pathology, Amman, Jordan, April 2011.

7. *Updates in Oxidative Stress and Ovarian Cancer*. Modern Technology Application in Pathology Conference, Amman, Jordan, July 22 – August 1, 2010.

8. *The Role of p53 in the Pathogenesis of Keloids*. International Meeting on Mechanisms Involved in Tissue Repair and Fibrosis: Role of the Microfibroblast (Differentiation and Apoptosis), Lyon, France, December 1997.

Local/Regional

1. *The Role of Oxidative Stress in the Pathogenesis of Ovarian Cancer*. Joint Annual Reproductive Sciences Retreat, Departments of Obstetrics and Gynecology, Wayne State University School of Medicine and The University of Toronto; and Annual Michigan Alliance for Reproductive Technologies and Sciences (MARTS) Meeting at Wayne State University, Detroit, MI, October 2017. Retreat

2. *Invited Guest Speaker*. Tumor Microenvironment Section, Karmanos Cancer Center, Detroit Medical Center/Wayne State University School of Medicine, Detroit, MI, June 2016.

3. *Molecular Biological Procedures*. C.S. Mott Center for Human Growth and Development, Division of Reproductive Endocrinology and Infertility Laboratory Techniques Summer Course, Wayne State University School of Medicine, Detroit, MI, September 2015.

4. *New Insights into Pathogenesis of Ovarian Cancer*. The C.S. Mott Center Summer Reproductive Sciences Technology Course, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, July 2014.

5. *The Role of Oxidative Stress in the Pathogenesis of Pro-Fibrotic Gynecologic Disorders*. 4th Annual Scientific Retreat, The C.S Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2014.

7. *Release of Substrates, Cofactors, and Products of Nitric Oxide Synthase Are Altered during Oocyte Aging*. 4th Annual Scientific Retreat, The C.S Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2014.

7. *The Impact of Myeloperoxidase on Metaphase II Mouse Oocyte Quality*. 4th Annual Scientific Retreat, The C.S Mott Center for Human Growth and Development, Department

of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, May 2014. **First Prize Award**

8. *Differential Expression of Glutathione Peroxidase and Glutathione Reductase in Chemoresistant Epithelial Ovarian Cancer Cells*. The Michigan Alliance for Reproductive Technologies and Science (MARTS), Fourth Annual Research Symposium, University of Michigan, Ann Arbor, MI, May 2013.

9. *The Role of Oxidative Stress in the Pathophysiology of Gynecologic Fibrotic Disorders: Postoperative Adhesions, Fibroids, Endometriosis, and Ovarian Cancer*. 1st Annual Scientific Retreat of The C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Southfield, MI, May 2011.

10. *New Insights in Ovarian Cancer Screening*. Department of Obstetrics and Gynecology Wayne Day: New Frontiers in the Treatment of Gynecologic Cancer, Wayne State University School of Medicine, Detroit, MI, December 2010.

11. *Molecular Characterization of Adhesion and Peritoneal Fibroblasts*. Adhesion Mini Symposium, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, March 2001.

12. *Multiplex RT/PCR Technique, Concept and Application*. Center for Biomedical Research, College of Art and Sciences, Oakland University, Rochester, MI, May 1999.

13. *Techniques for Characterizing and Manipulating DNA from the Basis of Much of Modern Biomedical Research*. Department of Chemistry, Oakland University, Rochester, MI, January 1999.

14. *Bcl-2/Bax Ratio as a Measure of the Rate of Apoptosis in Keloid Fibroblasts*. Oxford Biomedical Research Inc., Oxford, MI, January 1998.

15. *PCR Techniques, Concepts and Applications*. Howard Hughes Research Program, Oakland University, Rochester, MI, May 1998.

16. *Multiplex RT/PCR Technique, Concept and Application*. Center for Biomedical Research, College of Art and Sciences, Oakland University, Rochester, MI, May 1997.

17. *Application of RT/PCR*. Department of Chemistry, Oakland University, Rochester, MI, June 1994.

Invited Seminars and Grand Rounds

1. *New Insights into the Pathogenesis of Post-Operative Adhesions Development*. Department of Obstetrics and Gynecology Grand Rounds, Georgia Regents University, Augusta, GA, January 2017.

2. *Novel Innovative Targets for Ovarian Cancer Therapy*. Cancer Center Seminar, Georgia Regents University, Augusta, GA, January 2017.

3. *The Role of Oxidative Stress in the Pathogenesis of Pro-Fibrotic Gynecologic Disorders*. Augusta Research Day, Department of Obstetrics and Gynecology Grand Rounds, Georgia Regents University, Augusta, GA, June 2013.

4. *Dichloroacetate Induces Apoptosis of Epithelial Ovarian Cancer Cells Through the Inhibition of Oxidative Stress Enzymes*. SGI-SMFM Scientific Meetings Abstract Presentations, Department of Obstetrics and Gynecology Grand Rounds, Wayne State University School of Medicine, Detroit, MI, February 2010.

5. *PCR Techniques Concepts and Clinical Applications*. Clinical Fellows Seminar, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, March 2000.

6. *The Role of p53 and Apoptosis in the Pathogenesis of Keloids*. Department of Obstetrics and Gynecology Grand Rounds, Wayne State University School of Medicine, Detroit, MI, July 1998.